Charles Rodolphe
Brupbacher Foundation

Breakthroughs in Cancer Research and Therapy

Edited by Nancy E. Hynes & Lukas Sommer

Ponte Press 2019

Breakthroughs in Cancer Research and Therapy 2019
Nancy E. Hynes, Lukas Sommer (Eds.)
Ponte Press Verlags GmbH
D-44892 Bochum
ISBN 13: 978-3-920328-79-9

Impressum
Charles Rodolphe Brupbacher Stiftung
Wissenschaftliches Sekretariat
Bahnhofstrasse 24
P.O. Box
CH-8024 Zürich
Schweiz

Layout
Anke Redegeld
Lisa Marxt, lic. phil.

Druck
J. E. Wolfensberger AG, Birmensdorf

Klimaneutral
Druckprodukt
ClimatePartner.com/11664-1810-1002

FSC
www.fsc.org
MIX
Paper from responsible sources
FSC® C007616

Inhalt

Contents

Programm des Wissenschaftlichen Symposiums 2019

Program of the Scientific Symposium 2019

Charles Rodolphe Brupbacher Symposium 2019
Breakthroughs in Cancer Research and Therapy

TUESDAY, JANUARY 29, 2019

19:00 – 20:00
Charles Rodolphe Brupbacher Public Lecture
Anne Müller
Mikrobielle Infektionen als Ursache von Krebs

ETH Swiss Federal Institute of Technology, Main Building,
Audi Max (HG F 30) Rämistrasse 101, 8006 Zurich

WEDNESDAY, JANUARY 30, 2019

12:00 – 13:30
Registration / Sandwich Lunch

13:30 – 15:00
Cancer Cell Heterogeneity
Chair: Lukas Sommer

Andreas Trumpp
A single cell view on stem cells and cancer

Alain Puisieux
Cellular pliancy and breast cancer heterogeneity

Nicola Aceto
Heterogeneity and vulnerabilities of circulating tumor cells

15:00 – 15:30
Coffee break

15:30 – 17:30
Genetics and Epigenetics
Chair: Lauri Aaltonen

Peter Lichter
From (epi) genomic profiling to clinical applications

Jean-Philippe Theurillat
Towards the understanding of genetically-defined subtypes of prostate cancer

Nada Jabado
Oncohistones: how to turn the cell's symphony into non-harmonic rap

G. Steven Bova
Evolution of lethal metastatic cancer at primary and metastatic sites: Can we identiy what is important?

17:45 – 21:30
Posters, Beer and Pretzels
Evening for all participants

THURSDAY, JANUARY 31, 2019

08:00 – 08:30
Registration and Coffee

08:30 – 10:00
Big Data in Epidemiology and Cancer Research
Chair: Sir Alex Markham

Richard J. Gilbertson
Multi-organ mapping of cancer origins

Ian Tomlinson
Inherited factors that influence cancer and cancer risk behaviour

Serena Nik-Zainal
Insights from cancer genome sequencing: Utilising the totality of mutagenesis for clinical purposes

10:00 – 10:30
Coffee break

10:30 – 12:00
Signalling Pathways in Response & Resistance to Cancer Therapy
Chair: Nancy E. Hynes

Michael N. Hall
Mechanisms of Evasive Resistance in HCC

Daniel S. Peeper
Towards rational combinatorial cancer treatment - a functional genomics approach

Gerhard Christofori
Regulatory circuits in EMT, cell plasticity and cancer metastasis

12:00 – 13:30
Lunch and Coffee

13:30 – 15:00
Cancer Metabolism/Autophagy
Chair: Josef Jiricny

Sean Morrison
Lactate exchange promotes oxidative stress resistance and melanoma metastasis

Pierre Close
Codon-specific mRNA translation regulation in cancer

Matthew Vander Heiden
Role of metabolism in supporting tumor progression

15:00 – 15:30
Coffee Break

15:30 - 17:00
Tumour Microenvironment and Metastasis
Chair: Miriam Merad

Karin de Visser
Dissecting tumor-genotype/immunophenotype relationships in metastatic breast cancer

Johanna Joyce
Exploring and exploiting the tumour microenvironment

Paul Frenette
Cancer regulation by the bone marrow microenvironment

17:15 – 18:30
Charles Rodolphe Brupbacher Prize for Cancer Research 2019
Award Ceremony
Public Event

18:30 – 19:00
Apéro

FRIDAY, FEBRUARY 1, 2019

08:00 – 08:30
Coffee

08:30 – 10:00
Breakthroughs in Cancer Therapy
Chair: Markus Manz

Antoni Ribas
Overcoming resistance to PD-1 blockade therapy

Andrew W. Roberts
Targeting BCL2 as therapy for haematological malignancies: from concept to emerging clinical applications

Peter Hillmen
Curing CLL: a glimpe into the future of cancer treatment?

10:00 – 10:30
Coffee break

10:30 – 11:30
Breakthroughs in Cancer Biology
Chair: Holger Moch

Bernd Bodenmiller
Highly multiplexed imaging of tissues with subcellular resolution by imaging mass cytometry

Nicola Valeri
Patient-derived organoids: promises, hurdles and potential clinical applications

11:30 – 12:00
Young Investigator Awards
Nancy Hynes and Lukas Sommer
Public Event

Preisverleihung

Charles Rodolphe Brupbacher Preis für Krebsforschung 2019

Award Ceremony

Charles Rodolphe Brupbacher Prize for Cancer Research 2019

Charles Rodolphe Brupbacher Preis für Krebsforschung 2019

Die Stiftung verleiht alle zwei Jahre den Charles Rodolphe Brupbacher Preis für Krebsforschung an Wissenschaftler, die auf dem Gebiet der Grundlagenforschung hervorragende Leistungen erbracht haben. Die Preisverleihung findet statt im Rahmen eines internationalen wissenschaftlichen Symposiums, an dem auch der öffentliche Charles Rodolphe Brupbacher Vortrag gehalten wird.

Der Preis für das Jahr 2019 wird verliehen an:

Michael N. Hall, Basel

Charles Rodolphe Brupbacher Prize for Cancer Research 2019

Biennially, the Foundation bestows the Charles Rodolphe Brupbacher Prize for Cancer Research upon a scientist who has made extraordinary contributions to basic oncological research.
The Award Ceremony takes place within the framework of a Scientific Symposium, which includes the Charles Rodolphe Brupbacher Public Lecture.

The recipient of the 2019 Award is:

Michael N. Hall, Basel

Begrüssung

Prof. Dr. Michael O. Hengartner, Rektor der Universität Zürich

Mendelssohn: Lied ohne Worte für Cello und Klavier, Op. 109

Preisverleihung

Laudatio
Michael N. Hall, PhD
durch
Nancy E. Hynes, PhD

Tschaikowsky: Nocturne für Cello und Klavier, Op. 19 Nr. 4

Referat des Preisträgers
Michael N. Hall, PhD

Fauré: Elegie für Cello und Klavier, Op. 24

Schlussworte

Piazolla: Oblivion arrangiert für Cello und Klavier

Apéro

Introduction

Prof. Dr. Michael O. Hengartner, Rector of the University of Zurich

Mendelssohn: Song without Words for Cello and Piano, Op. 109

Award

Laudatio
Michael N. Hall, PhD
by
Nancy E. Hynes, PhD

Tschaikowsky: Nocturne for Cello and Piano, Op. 19 Nr. 4

Acceptance Speech
Michael N. Hall, PhD

Fauré: Elegy for Cello and Piano, Op. 24

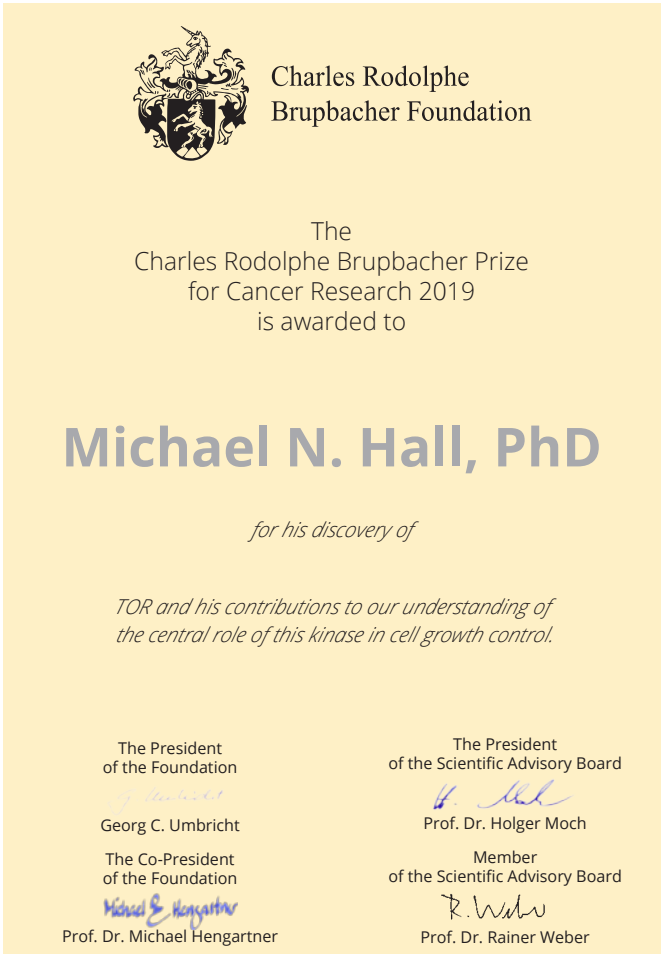
Final address

Piazolloa: Oblivion arranged for Cello and Piano

Apéro

Laudatio

Nancy E. Hynes



On behalf of the Charles Brupbacher Foundation, I am pleased to write the laudatio for Professor Michael N. Hall, the winner of the 2019 Charles Rodolphe Brupbacher Prize for cancer research. Michael N. Hall is being given the award for his discovery of TOR (target of rapamycin) and his contributions to our understanding of the central role of this conserved kinase in cell growth control. His scientific achievements since the discovery of TOR more than 25 years ago have been essential for the development of TOR inhibitors, which are now used for cancer treatment.

Mike Hall joined the Biocenter at the University of Basel in the late 1980s after obtaining his PhD from Harvard and doing postdoctoral training at UCSF in California. During this period, the major focus of his work was to learn how proteins localize to particular cellular regions, using bacteria and yeast as model systems. At the Biocenter, Mike was hosting an MD/PhD student in his lab who was interested in how drugs work in humans. At the same time, Sandoz was working on cyclosporin A (CyA) and FK506, both of which have immunosuppressive activity and were used for treatment of rheumatoid arthritis and other diseases. Rapamycin had similar activity, although it was not in clinical usage at that time. Little was known about the mechanism of action of these drugs, however, it was known that they blocked the nuclear import of a signal downstream of the T-cell receptor. Considering Mike's interest in nuclear protein import, he thought that perhaps by studying these drugs, he could unravel a signaling pathway that connected the cytoplasm of the cell to the nucleus.

Mike's lab showed that one of these drugs, rapamycin, blocked proliferation of yeast cells. Working with yeast has many advantages when it comes to doing genetics. Following on this discovery, it was relatively straightforward to identify rapamycin-resistant mutants and to map the genes responsible for the phenotype, which were dubbed TOR1 and TOR2 for "target of rapamycin." The paper describing these findings was published in 1991 and has been cited more than 1000 times since then. Next, performing a clever genetic screen using the rapamycin-resistant cells, they isolated the TOR genes based on the functional regain of rapamycin sensitivity. Mechanistically, the immunosuppressive macrolide antibiotics rapamycin and FK506 form complexes with FKBP and it is this complex that binds and inhibits TOR resulting in blockade of cellular division. A paradigm shifting discovery was made a few years later by the Hall lab, when they found that the anti-proliferative effects of TOR inhibition were not due to direct effects on cell cycle regulators. Experimentally, this was based on the finding that TOR inhibition did not cause yeast cells to increase in cell size, which is what inhibitors of cell cycle proteins

were known to cause. Accordingly, they proposed that there must be a distinction between cell growth and cell division, and importantly cell growth must be a regulated process. This was a completely novel concept at that time and was not immediately accepted. However, over the past 20 years what was initially met with skepticism has become textbook dogma. Indeed, we now know that TOR, and its mammalian homolog mTOR, are evolutionarily conserved phosphatidylinositol 3-kinase (PI3K)-like serine/threonine protein kinases and that TOR is the central regulator of cell growth via its ability to respond to nutrients and growth factors.

I will next comment on what I think contributes to making Mike Hall`s scientific work special and deserving of this year's Brupbacher prize. Today the concept of using a model organism like yeast to unravel the mechanisms of action of human drugs is well accepted, but this was not the case 30 years ago when Mike began his work on rapamycin. Science functions best by allowing researchers to take seemingly wild approaches to uncover mechanisms underlying the phenomena they observe and that drive their scientific curiosity. This is certainly true of Mike's work on TOR, which started with an interesting observation of yeast mutants that failed to stop growing in response to treatment with a drug that had immunsuppressive activity in humans.

It is also essential to discuss the importance of Mike`s findings for medicine, particularly cancer. TOR is a central kinase on the PI3 kinase/AKT kinase pathway. In this short laudatio, it is impossible to describe all the beautiful work from the Hall lab, as well as many others working on TOR, that has led to our current understanding of this essential signaling pathway. We know that the PI3K/AKT/TOR pathway is constitutively activated by different mechanisms in most solid tumors and much effort has gone into developing inhibitors for each kinase. I will concentrate on how TOR inhibitors have significantly changed the management of patients with the diseases tuberous sclerosis (TS) and cancer. TS is a multiorgan genetic disease that causes benign tumors in brain, kidney and other organs. Although benign, tumor growth in vital organs can have drastic effects. Mutations in genes encoding TOR inhibitory proteins underly this disease and positive results in clinical trials resulted in the 2010 approval of the TOR inhibitor everolimus for distinct manifestations of TS. Considering solid cancers, in 2009 everolimus was approved for treatment of patients with advanced kidney cancer, in 2011 for advanced pancreatic neuroendocrine tumor treatment, and in 2012 for women with estrogen receptor positive advanced breast cancer. These approvals were based on the clinical findings that blocking TOR significantly prolonged disease-free patient survival.

In conclusion, I would like to go back to Mike's first goal in the 1980s, which was to uncover a cytoplasmic signaling pathway that converged on the nucleus. This goal has clearly been achieved. When TOR was first described as a target of rapamycin it could not be linked to any other proteins. Since then work from Mike's and other labs has revealed that TOR activates anabolic processes like ribosome biogenesis and protein synthesis, and inhibits catabolic processes including autophagy, to control cell growth. Since the major upstream activators of TOR, PI3K and AKT are often constitutively active in cancer, TOR inhibition is being actively pursued and has already had clinical success. Considering the high conservation of the PI3K/AKT/TOR pathway, I am convinced that we will be hearing about other indications for TOR inhibitors in the future.

Michael N. Hall

Summary Curriculum vitae

Copyright: Biozentrum / Matthew Lee

Appointment Professor, University of Basel

Address Biozentrum, University of Basel
Klingelbergstrasse 70
CH-4056 Basel, Switzerland

Date of Birth June 12, 1953.

Education

1981-1984	Postdoctoral Fellow, University of California, San Francisco, California
1981	Ph.D., Harvard University, Boston, Massachusetts
1976	B.S. with Honors, University of North Carolina, Chapel Hill, North Carolina

Academic Appointments/Affiliations

1992-present	Professor, University of Basel, Switzerland
2013-2016	Vice Director, Biozentrum, University of Basel
2002-2009	Vice Director, Biozentrum, University of Basel
2002-2008	Chairman, Division of Biochemistry, University of Basel
1995-1998	Chairman, Division of Biochemistry, University of Basel
1984-1987	Assistant Research Biochemist/Principal Investigator, Department of Biochemistry & Biophysics, University of California, San Francisco
1981-1984	Helen Hay Whitney Fellow, Department of Biochemistry & Biophysics, University of California, San Francisco
1981	Association pour le Développement de l'Institut Pasteur (ADIP) Fellow, Unité de Génétique Moléculaire, Institut Pasteur, France
1979-1981	Harvard University Traveling Scholar, NCI, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland
1976-1979	NIH Training Grant Fellow, National Research Service Award, Department of Microbiology &Molecular Genetics, Harvard Medical School, Boston, Massachusetts
1975-1976	Research Assistant with Marshall Edgell and Clyde Hutchison, Department of Bacteriology & Immunology, University of North Carolina, Chapel Hill, North Carolina

Awards & Honors

1981	Association pour le Développement de l'Institut Pasteur (ADIP) Fellowship
1981-1984	Helen Hay Whitney Fellowship
1982	Litton Advanced Technology Achievement Award
1987	Cuban Academy of Science Invited Lecturer, Havana, Cuba
1995	Member of the European Molecular Biology Organization (EMBO), elected
2003	Cloëtta Prize for Biomedical Research, Prof. Dr. Max Cloëtta Foundation
2004	Susan Swerling Lecture, Harvard Medical School
2008-2016	Swiss National Science Foundation Research Council
2009	Louis-Jeantet Prize for Medicine, Fondation Louis-Jeantet
2009	Runnström Lecture, University of Stockholm
2009	Fellow of the American Association for the Advancement of Science (AAAS), elected
2010	Mendel Lecture, Czech Academy of Arts and Sciences, Brno, Czech Republic
2011	Allan C. Wilson Lectures, University of California, Berkeley
2012	EMBO Lecture, Oslo, Norway
2012	Marcel Benoist Prize for Sciences or Humanities, Marcel-Benoist-Stiftung
2013	Swiss Academy of Medical Sciences, elected
2013	Jesus Montoliu Lecture, The Biomedical Research Institute of Lleida, Spain
2013	Christian de Duve Lecture (Inaugural), Université Catholique de Louvain, Brussels

2014	University Visiting Professorship, The Hebrew University, Jerusalem
2014	Sir Hans Krebs Medal, Federation of European Biochemical Societies (FEBS)
2014	Breakthrough Prize in Life Sciences
2014	Member of the National Academy of Sciences USA, elected
2015	UCSF Alumni Excellence Award
2015	Canada Gairdner International Award for Biomedical Research, Gairdner Foundation
2016	Benning Lecture, University of Utah, USA
2016	Thomson Reuters Citation Laureate
2016	Doctor *honoris causa*, University of Geneva
2016	Debrecen Award for Molecular Medicine, University of Debrecen, Hungary
2016	Distinguished Investigator, Instituto de Biomedicina de Sevilla (IBiS), Spain
2017	Szent-Györgyi Prize for Progress in Cancer Research, NFCR
2017	Albert Lasker Basic Medical Research Award, Albert and Mary Lasker Foundation
2018	Genome Valley Excellence Award, BioAsia, Hyderabad, India
2018	King Lecture, Clare Hall, University of Cambridge, UK

Professional Memberships & Activities

2008-2013	Editorial Board, FEBS Journal
2008-2012	Review Panel, National Center of Research (NCCR) in Structural Biology, Switzerland
2008	SNSF Ambizione Grants evaluation panel
2010	External Scientific Advisory Board, Faculty of Medicine, University of Geneva
2011	Scientific Committee, Louis-Jeantet Foundation, Geneva
2011	Editorial Board, The EMBO Journal
2011-2015	Review Panel, National Center of Research (NCCR) in Chemical Biology, Switzerland
2011	Scientific Advisory Board, Centre for Biological Signalling Studies (BIOSS), Germany
2012-2015	EMBO Young Investigator Programme (YIP) Selection Committee, Heidelberg
2012	Chair, Cell Metabolism and Cell Homeostasis Symposium, Dresden, Germany
2012-2017	Scientific Advisory Board, Max-Planck Institute for Biochemistry, Martinsried, Germany
2012	Co-organizer, Les Treilles Conference: Growth Regulation by the TOR Pathway, France
2012	Co-organizer, 2012 Louis-Jeantet Symposium, Geneva, Switzerland
2012	Scientific Advisory Board, PIQUR Therapeutics
2013	Instructor, FEBS-EACR advanced course on Signal Transduction, Spetses, Greece
2014	Selection Committee, Breakthrough Prize in Life Sciences Foundation
2014-2018	Scientific Council, de Duve Institute, Brussels, Belgium
2014	Board of Trustees, Louis-Jeantet Foundation, Geneva
2014	External Review Committee, Okinawa Institute of Science and Technology, Japan
2014	Roche Commissions, research as architecture, architecture as research, with J. Herzog
2014	External Review Committee, Okinawa Institute of Science and Technology, Japan
2015	Co-organizer, International Abcam Conference: PI3K-like Protein Kinases, Milan, Italy
2015	Instructor, FEBS advanced course on Signal Transduction & Cancer, Spetses, Greece
2015-2019	Scientific Advisory Board, Navitor Pharmaceuticals, Inc.
2015	Editorial Board, ScienceMatters
2015	American Association for Cancer Research (AACR)
2016	European Research Council (ERC) Advanced Grants evaluation panel
2016	Executive Board, Personalized Health Basel
2016	International Scientific Advisory Board, Cambridge Institute for Medical Research
2016	European Association for Life Sciences (EALS) Board
2016	Keynote Lecture, 9th International Symposium on AMPK, Xiamen, China
2017-2019	Advisory Board, Marcel Benoist Foundation, Bern
2017	Scientific Advisory Board, Swiss Institute for Basic Cancer Research (ISREC)
2017-2020	European Molecular Biology Organization (EMBO) Council
2017-2020	Editorial Board, Current Opinion in Cell Biology
2018	Co-organizer, EMBO at Basel Life Conference: Molecules in Biology and Medicine
2018	Selection Committee (Chair), Szent-Györgyi Prize, NFCR National Foundation for Cancer
2018	International Advisory Board, Melbourne bid for joint IUBMB/ComBio meeting

Keynote Lectures

2012	Keynote Lecture, Symposium of the Zürich Center for Integrative Human Physiology
2012	Inaugural Lecture, Instituto de Biología Funcional y Genómica, Salamanca, Spain
2014	Keynote Lecture, FASEB Research Conference, Steamboat Springs, USA
2014	Keynote Lecture, Israeli Society for Cancer Research, Haifa, Israel
2014	Sir Hans Krebs Lecture, FEBS-EMBO Congress, Paris, France
2014	Keynote Lecture, EMBO/EMBL Symposium, Heidelberg, Germany
2015	Honors Program Lecture, New York University School of Medicine, NYC
2015	Keynote Lecture, International TSC Research Conference, Windsor, England
2015	Keynote Lecture, 40th European Symposium on Hormones and Cell Regulation, France
2017	Lola and John Grace Distinguished Lecture in Cancer Research, Lausanne (EPFL
2017	Karl Wilhelm von Kupffer Lecture, The International Liver Congress, Amsterdam
2017	Keynote Lecture, Gordon Research Conference, Integrative Biology of Aging
2017	Plenary Lecture, ASCB-EMBO Congress, Philadelphia, USA
2018	EMBL Distinguished Visitor Lecture, Heidelberg, Germany
2018	Keynote Address, BioAsia 2018, Hyderabad, India
2018	Keynote Lecture, TOR de France, Nice, France
2018	Keynote Lecture, TOR de France, Nice, France
2018	Keynote Lecture, Roche Continents, Salzburg, Austria
2018	Keynote Address, Toyama Symposium, Japan

Publications

http://www.biozentrum.unibas.ch/research/groups-platforms/publications/unit/hall

mTOR signaling in growth and metabolism

Michael N.Hall

Cell division, growth and death are the most basic, fundamental features of biology. Research on cell growth started in earnest after mechanisms controlling cell division and cell death were already well elucidated. The turning point in our understanding of cell growth came in 1991 with the discovery of TOR (Target of Rapamycin), the key component of the cell growth control system. TOR is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, and cellular energy. TOR was originally discovered in yeast but is conserved in all eukaryotes including plants, worms, flies, and mammals. In mammals, TOR is known as mTOR. The discovery of TOR led to a fundamental change in how one thinks of cell growth. It is not a spontaneous process that just happens when building blocks (nutrients) are available, but rather a highly regulated, plastic process controlled by TOR-dependent signaling pathways. TOR controls cell growth by activating anabolic processes such as ribosome biogenesis, and protein, nucleotide and lipid synthesis, and by inhibiting catabolic processes such as autophagy. TOR is found in two structurally and functionally distinct multiprotein complexes, TORC1 and TORC2. The two TOR complexes, like TOR itself, are highly conserved. Thus, the two TOR complexes constitute an ancestral signaling network conserved throughout eukaryotic evolution to control the fundamental process of cell growth. As a central controller of cell growth, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, allograft rejection, obesity, and diabetes. While the role of TOR in controlling growth of single cells is relatively well understood, the challenge now is to understand the role of TOR signaling in disease and in coordinating and integrating overall body growth and metabolism in multicellular organisms.

Bisherige Preisträger
Previous Laureates

1993

Arnold J. LEVINE
Department of Molecular Biology, Lewis Thomas Laboratory, Princeton University, Princeton, NJ, USA
«Functions of the p53 Gene and Protein»
David P. LANE
Cancer Research Campaign Laboratories, Department of Biochemistry, University of Dundee, Dundee, Scotland
«The p53 Pathway, Past and Future»

1995

Alfred G. KNUDSON
Fox Chase Cancer Center, Philadelphia, PA, USA
«Hereditary Cancer»
Robert A. WEINBERG
Whitehead Institute for Biomedical Research, Department of Biology, MIT, Cambridge, MA, USA
«Genes and Cancer»

1997

Laurent DEGOS
Institut Universitaire d'Hématologie, Hôpital Saint Louis, Paris, France
«Differentiation Therapy of Cancer»
Zhen-yi WANG
Shanghai Institute of Hematology, Rui-Jin Hospital Shanghai, Second Medical University, Shanghai, China
«Treatment of Acute Promyelocytic Leukemia with All-Trans Retinoic Acid. A Model of Differentiation Therapy in Cancer»

1999

George KLEIN
Microbiology and Tumor Biology Center (MTC)
Karolinska Institute, Stockholm, Sweden
«Cancer and the New Biology»
Harald ZUR HAUSEN
Deutsches Krebsforschungzentrum, Heidelberg, Germany
«Cancer Causation by Viruses»

2001

Brian DRUKER
Oregon Health Sciences University, Portland, OR, USA
«STI571: A Tyrosine Kinase Inhibitor for the Treatment of CML – Validating the Promise of Molecularly Targeted Therapy»

2003

Rudolf JAENISCH
Whitehead Institute for Biomedical Research, Department of Biology, MIT, Cambridge, MA, USA
«Nuclear Cloning and the Reversibility of Cancer»
Erwin F. WAGNER
Institute of Molecular Pathology, Vienna, Austria
«Unravelling the Functions of AP-1 (Fos/Jun) in Mouse Development and Disease»

2005

Mariano BARBACID
Centro Nacional de Investigaciones Oncológicas, Madrid, Spain
«The Molecular Bases of Human Cancer: a 25 Year Journey»
Klaus RAJEWSKY
The CBR Institute for Biomedical Research,
Harvard Medical School, Boston, MA, USA
«The Janus Face of Antibody Formation: Protective Function and Tumor Risk»

2007

Lloyd J. OLD
Ludwig Institute for Cancer Research, New York, NY, USA
«Contributions to the Field of Cancer Immunology»
Robert D. SCHREIBER
Department of Pathology and Immunology, Washington University School of Medicine, St.Louis, MO, USA
«Cancer Immunoediting: Deciphering the Complex Interaction Between Immunity and Developing Tumors»
Mark J. SMYTH
Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
«Extrinsic Tumor Suppression by Innate and Adaptive Immunity»

2009

Nubia MUÑOZ
National Cancer Institute, Bogota, Colombia
«From Causality to Prevention: The Case of Cervical Cancer»
Sir Richard PETO
Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom
«The Absolute Benefits of Anti-cancer Drugs and of Tobacco Control»

2011

Jan HOEIJMAKERS
Department of Genetics, Erasmus Medical Center, Rotterdam, The Netherlands
«DNA Damage and its Impact on Cancer, Aging and Longevity»
Bert VOGELSTEIN
The Sidney Kimmel Comprehensive Cancer Center
Johns Hopkins University, Baltimore, MD, USA
«The Genetic Basis of Human Cancer and its Implications for Patient Management»

2013

Michael KARIN
Department of Pharmacology
University of San Diego, California, USA
«Inflammation and Cancer: Effects, Mechanisms and Therapeutic Implications»

2015

Joan MASSAGUÉ
Sloan Kettering Institute
Memorial Sloan Kettering Cancer Center
«Molecular Basis of Metastasis»
Irving L. WEISSMAN
Institute for Stem Cell Biology and Regnerative Medicine
Stanford University
«The view from stem cell land: Stem cell biology in regeneration and cancer»

2017

Laurence ZITVOGEL
Institut Gustave Roussy, Villejuif, France
«The role of immunogenic cell death and the gut microbiota in cancer treatment»
Guido KROEMER
Institut Gustave Roussy, Villejuif, France
«Cancer cell stress: Cell-autonomous and immunological considerations»
Sir Adrian Peter BIRD
Wellcome Trust Centre for Cell Biology,
The University of Edinburgh, UK
«DNA methylation patterns and cancer»

Abstracts
Eingeladene Redner

Abstracts
Invited Speakers

Öffentlicher Vortrag:
Mikrobielle Infektionen als Ursache von Krebs

Anne Müller
Institut für molekulare Krebsforschung, Universität Zürich

Krebs gilt im Allgemeinen als genetische Erkrankung, die durch vererbte oder neu erworbene Veränderungen des Erbguts (der DNA), den sogenannten Mutationen verursacht wird. Beispiele hierfür sind manche Formen von Brustkrebs, oder der bösartige schwarze Hautkrebs, die durch vererbte Punktmutationen und UV-induzierte Schädigung der DNA entstehen. Seit ca. 20 Jahren ist jedoch klar, dass ein substantieller Anteil der jährlich neu diagnostizierten Tumorerkrankungen nicht durch Vererbung oder durch physikalische oder chemische DNA Schädigung, sondern durch Infektionen ausgelöst werden. Man schätzt diesen Anteil inzwischen auf ca. 20% aller Tumorerkrankungen. Ein grosser Teil der ca. 2 Mio. jährlich neu diagnostizierten, infektionsabhängigen Tumoren geht auf das Konto von nur vier Erregern. Drei davon sind Viren, nämlich einerseits das humane Papillomvirus, welches ca. 600'000 Fälle von Gebärmutterhalskrebs jährlich auslöst, und andererseits die Hepatitisviren B und C, welche zusammen genommen eine ähnlich hohe Zahl von Leberkarzinomen auslösen. Beim vierten numerisch dominanten krebsauslösenden Keim handelt es sich um ein Bakterium, Helicobacter pylori, welches den menschlichen Magen besiedelt und dort Magenkrebs verursachen kann. Von der Erstinfektion bis zur Krebsdiagnose vergehen häufig Jahrzehnte, und Kofaktoren wie Alkohol, salzreiche Ernährung und erbliche Faktoren müssen hinzukommen bevor Krebs entsteht. Nicht alle akut oder auch chronisch Infizierten entwickeln Krebs. Allerdings steht fest, dass das Risiko, an Gebärmutterhals-, Magen- oder Leberkrebs zu erkranken in Nicht-infizierten mit unter 10% gering ist. Aus diesem Grund geht man davon aus, dass die Entwicklung von Impfstoffen gegen die zugrundeliegende Infektion ein wirksames Mittel sein müsste, die Inzidenzen der genannten Krebsformen zu reduzieren. Tatsächlich wird mit Hochdruck an derartigen Impfstoffen geforscht. Gegen das Papillomvirus steht inzwischen ein wirksamer Impfstoff zur Verfügung, der in Industrieländern flächendeckend angeboten wird. Das Gleiche gilt für das Hepatitis B Virus, gegen welches schon seit Jahrzehnten erfolgreich geimpft wird. Beide Impfstoffe werden rekombinant hergestellt und gelten als ausgesprochen sicher und wirksam. Gegen Hepatitis C und Helicobacter pylori ist bisher jeweils kein Impfstoff verfügbar und es ist unklar, ob es in diesen Fällen jemals gelingen wird, einen Impfstoff zu entwickeln. Stattdessen setzen Länder, die es sich leisten können, auf regelmässige Kontrolle und strikte Eradikation, im Fall von Helicobacter pylori mit Hilfe von Antibiotika. Länder wie die Schweiz, in denen Neuinfektionen mit Helicobacter pylori seit vielen Jahren rückläufig sind, weisen nur noch eine geringe Magenkrebsrate auf. In diesen Ländern steigt wiederum der Anteil der Bevölkerung, der unter Allergien und Autoimmunerkrankungen leiden; beide Formen von immunologischen Erkrankungen stehen im Verdacht, durch Helicobacter pylori effizient unterdrückt zu werden. Helicobacter pylori besiedelt den menschlichen Magen seit ca. 100'000 Jahren und hat sich im Lauf seiner Ko-Evolution mit dem Menschen zu einem erstklassigen Manipulator des menschlichen Immunsystems entwickelt, der allergenspezifische Immunantworten, wie auch die gegen ihn selbst gerichtete Immunabwehr effizient unterdrückt. Nicht alle Keime, die den Menschen befallen oder besiedeln sind Krankheitserreger. Seit einigen Jahren ist sogar klar, dass eine gesunde Mikrobiota, also die Gesamtheit der Mikroben die den Menschen besiedeln, sogar entscheidend für eine erfolgreiche Tumorbehandlung ist. Dieses Forschungsgebiet steckt derzeit noch in den Kinderschuhen. Weitere grossangelegte klinische Studien sind notwendig, um für jeden Tumortyp aufzuklären ob und wie Mikroben das Geschehen beeinflussen.

A single cell view on stem cells and cancer

Andreas Trumpp
Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM) gGmbH, Heidelberg, Germany

Dormant hematopoietic stem cells (dHSCs) define the top of the hematopoietic hierarchy. The molecular identity of dHSCs and the mechanisms regulating their maintenance or exit from dormancy remain uncertain. Using single-cell RNA-seq analysis we show that the transition from dormancy towards cell cycle entry is achieved by a continuous up-regulation of biosynthetic activity. Low Myc activity and biosynthesis rates, but engagement of a vitamin A/retinoic acid (RA) program are characteristic for dHSCs. Our results highlight the impact of dietary vitamin A on the regulation of activation mediated stem cell plasticity (Cabezas-Wallscheid, et al., Cell 2017). In addition, we integrated flow cytometric, transcriptomic and functional data at single-cell resolution to quantitatively map early differentiation of human HSCs towards lineage commitment. During homeostasis, individual HSCs gradually acquire lineage biases along multiple directions without passing through discrete hierarchically organized progenitor populations. Instead, unilineage-restricted cells emerge directly from a "Continuum of LOw primed UnDifferentiated hematopoietic stem- and progenitor cells" (CLOUD-HSPCs). These data reveal a continuous landscape of human steady state haematopoiesis downstream of

HSCs and provide a basis for the understanding of hematopoietic malignancies (Velten et al., Nature Cell Biol. 2017).
Metastatic spread is a complex process initiated by the dissemination, seeding and engraftment of malignant cells in sites distant to the primary tumor. We have shown that metastasis-initiating cells (MICs) are present within circulating-tumor-cells (CTCs) in the blood stream of breast cancer patients. We are now using single cell RNAseq analysis of CTCs isolated from metastatic luminal breast cancer patients before, during and after therapy (i.e. CDK4/6 inhibitor palbociclib) to determine the dynamic changes of the cellular heterogeneity during therapy, remission and relapse at the single cell level. Together with other multi-omics data we aim to identify novel drug resistance mechanisms directly from patient samples.

Cellular pliancy and breast cancer heterogeneity

Alain Puisieux
University of Lyon, Centre Léon Bérard, Lyon, France

The plasticity of cancer cells underlies their capacity to adapt to the selective pressures they encounter during tumor development. Aberrant reactivation of the epithelial-mesenchymal transition (EMT), a latent embryonic transdifferentiation program, promotes cancer cell plasticity and fuels tumor development and metastatic spread. Consistent with a prominent role in tumorigenesis, we have shown that EMT-inducing transcription factors of the TWIST and ZEB families act as genuine oncoproteins, fostering cell transformation and primary tumor growth by alleviating key oncosuppressive mechanisms and by providing cells with self-renewal propertiesè [1-3]. Recently, we have further demonstrated that EMT inducers are expressed in normal mammary stem cells and that their expression influences the entire natural history of breast tumorigenesis [4]. In contrast with differentiated cells, human mammary stem cells have the innate capacity to withstand an aberrant mitogenic activation. This property is based on an antioxidant program driven by the ZEB1 EMT inducer and the methionine sulfoxide reductase MSRB3. This pre-emptive program prevents the formation of oncogene-induced DNA damage, a major cause of genomic instability, and influences the emergence of cancer-associated events. Overall, our data suggest that malignant transformation of mammary stem cells does not hinge on genomic instability and indicate that intrinsic properties of the cell-of-origin determine the susceptibility to malignant transformation when subjected to an oncogenic insult, an emerging notion referred to as "cellular pliancy" [5] . Cellular pliancy is defined as the intrinsic capacity of a cell to adapt to a private oncogenic event. We propose that each discrete differentiation state within a given lineage is associated with a unique level of pliancy, which is epigenetically determined. This level relies upon the dynamic cooperation between the molecular and metabolic networks of the cell and the oncogenic event, and dictates the genomic landscape of human cancers.

1. Ansieau S, Bastid J, Doreau A, Morel AP, Bouchet BP, Thomas C, Fauvet F, Puisieux I, Doglioni C, Piccinin S, Maestro R, Voeltzel T, Selmi A, Valsesia-Wittmann S, Caron de Fromentel C, Puisieux A. Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. Cancer Cell, 14: 79-89, 2008.

2. Caramel J, Papadogeorgakis E, Hill L, Browne GJ, Richard G, Wierinckx A, Saldanha G, Hutchinson P, Tse G, Lachuer J, Puisieux A, Pringle JH, Ansieau S, Tulchinsky E. A switch in the expression of embryonic EMT-inducers drives the development of malignant melanoma. Cancer Cell, 24: 466-480, 2013.

3. Puisieux A, Brabletz T, Caramel J. Oncogenic roles of EMT-inducing transcription factors. Nature Cell Biology, 16: 488-494, 2014.

4. Morel AP, Ginestier C, Pommier RM, Cabaud O, Ruiz E, Wicinski J, Devouassoux-Shisheboran M, Combaret V, Finetti P, Chassot C, Pinatel P, Fauvet F, Saintigny P, Thomas E, Moyret-Lalle C, Lachuer J, Despras E, Jauffret JL, Bertucci F, Guitton J, Wierinckx A, Wang Q, Radosevic-Robin N, Penault-Llorca F, Cox DG, Hollande F, Ansieau S, Caramel J, Birnbaum D, Vigneron A, Tissier A, Charafe-Jauffret E, Puisieux A. A ZEB1-MSRB3 axis prevents oncogene-induced DNA damage in mammary stem cells and drives malignant transformation in absence of chromosomal instability. Nature Med, 23:568-578, 2017.

5. Puisieux A, Pommier RM, Morel AP, Lavial F. Cellular pliancy and the multistep process of tumorigenesis. Cancer Cell, 33: 164-172, 2018.

Heterogeneity and vulnerabilities of circulating tumor cells

Nicola Aceto
Department of Biomedicine, University of Basel and University Hospital Basel, Switzerland

Cancer patients that develop a metastatic disease are currently considered incurable. Mainly, this is due to a limited understanding of the molecular mechanisms that characterize the metastatic process, and the lack of effective metastasis-suppressing agents. The metastatic cascade begins with primary tumor cells entering the blood circulation, and it is followed by their extravasation at distant sites, where they form proliferative metastatic lesions. Cancer cells in circulation are referred to as circulating tumor cells (CTCs). Their isolation and characterization has recently become possible, and it has revealed highly unexpected features of the metastatic process. For instance, using a combination of microfluidics, single cell sequencing, molecular and computational biology, we understood that CTC clusters, rather than single CTCs, are highly efficient precursors of metastasis in breast and prostate cancer. More recently, we have gained fundamental insights into the biology of CTC clusters, linking their physical features to specific molecular changes that promote stemness and metastasis, allowing us to define new ways to suppress their metastatic potential. Further, we have begun to dissect CTC heterogeneity at the single cell level, revealing fundamental interactions that occur between CTCs and immune cells and that accelerate the metastatic process. Together, our latest findings support a model whereby CTCs form multicellular aggregates with each other as well as with immune cells to expand their metastatic potential. Targeting these interactions may lead to the development of new metastasis-suppressing agents.

From (epi) genomic profiling to clinical applications

Peter Lichter
German Cancer Research Center (DKFZ) Heidelberg, Germany

Large scale comprehensive genomic profiling - recently complemented by molecular profiling on epigenomic levels such as the methylome or chromatin structure - has greatly advanced our understanding of tumor genome biology. An emerging pattern is the frequent hi-jacking of regulatory DNA elements, such as enhancers and promoters, resulting in the deregulation of genes that drive oncogenesis, or the discovery of a previously unrecognized phenomenon of genomic instability termed chromothripsis, resulting in highly aberrant chromosomes. Elucidation of the respective pathomechanisms delineate novel vulnerabilities of tumors that are preclinically validated. Furthermore, genomic and epigenomic profiles have greatly contributed to the refinement of tumor classification schemes or even uncovered novel tumor entities. Examples from our activities within the International Cancer Genome Consortium (ICGC) will be presented. These studies have also triggered the establishment of clinical registry trials that are designed to explore the concept of precision oncology through molecular therapies targeting (epi)genomic alterations. On the basis of our current experience, future options for clinical translation of genome-based information will be discussed.

Towards the understanding of genetically-defined subtypes of prostate cancer

Tiziano Bernasocchi (1, 2)*, Geniver El Tekle (1, 2)*, Marco Bolis (1), Tanya Svinkina (3), Anna Rinaldi (1), Marita Zoma (1), Valentina Ceserani (1), Hana Janouskova (1), Peter Schraml (4), Guiseppina Carbone (1, 5), Andrea Alimonti (1, 5), Holger Moch (4), Steven A. Carr (3), Namrata D. Udeshi (3), Jean-Philippe P. Theurillat (1, 2, 5)+

* These authors contributed equally to this work.
+ Correspondence to: JeanP_Theurillat@ior.usi.ch

(1) Institute of Oncology Research, Oncology Institute of Southern Switzerland, Bellinzona, TI 6500, Switzerland. (2) University of Lausanne, Lausanne, VD 1011, Switzerland. (3) The Broad Institute of MIT & Harvard, Cambridge, MA 02142, USA. (4) Institute of Surgical Pathology, University Hospital Zurich, Zurich, ZH 8091, Switzerland. (5) University of Southern Switzerland, Lugano, TI 6900 Lugano, Switzerland

While the co-operation of cancer driver genes in tumorigenesis has been well-annotated, little is known about the interplay of driver mutations that never co-occur within the same cancer cells. The latter scenario has been identified in prostate cancer where recurrent gene fusions involving the oncogenic ERG transcription factor and point mutations in the ubiquitin ligase adaptor SPOP are strictly mutually exclusive. Nevertheless, the underlying basis of this observation is poorly understood. Here, we show that ERG and mutant SPOP – even though oncogenic on their own – are together synthetic lethal. At the molecular level both driver genes inhibit each other in a reciprocal manner. In ERG-driven tumors, wild type SPOP is required to dampen androgen receptor (AR) signaling and sustain ERG activity in part through its ability to degrade the bromodomain histone reader ZMYND11. Consequently, loss-of-function mutations in SPOP unleash excessive AR signaling and reduce ERG function. Conversely,

oncogenic androgen receptor signaling driven by mutant SPOP is repressed by ERG. The incompatibility of mutant SPOP and ERG may help to understand why SPOP mutant tumors frequently harbor gene deletions in the chromatin-modifying enzyme CHD1. We find that mutant SPOP promotes the generation of ERG rearrangements in a CHD1-dependent manner. Thus, CHD1 gene deletions may protect SPOP-mutant tumors from ERG-mediated lethality. Taken together, our findings reveal the existence of divergent and incompatible paths towards prostate cancer that converge on SPOP function.

Oncohistones: how to turn a cell's symphony into non harmonic rap?

Nada Jabado
Montreal Children's Hospital, McGill University, Research Institute of the McGill University Health Center, Montreal, QC, Canada

Recent studies have shown that chromatin-associated proteins and transcription factors have more somatic alterations than any other class of oncoproteins in childhood CNS tumors. Different Histone H3 genes (H3F3A, Hist3.1B) and variants (K27, G34, K36) can be affected with remarkable specific association between tumor location and type as well as the particular H3 residue or variant that is mutated. We have shown a high prevalence of H3 mutations in pediatric and young adult High Grade Astrocytoma, in sarcomas such as Giant Cell Tumors of the bone and chondroblastomas, and most recently, in Head and Neck Squamous Cell Carcinomas. These ground-breaking discoveries of oncohistones implicate a direct effect of epigenetic misregulation in oncogenesis. Here, we describe these epigenetic misfits and our knowledge of their effects, along with novel tools needed to study them. We will also discuss how we are harnessing synergies between the approaches of cancer genomics and chemical biology to help make sense of the pathogenesis of oncohistones and describe how oncohistones are promoting global redistribution of important epigenetic marks, seemingly hijacking our epigenome.

Evolution of lethal metastatic cancer at primary and metastatic sites: can we identify what is important?

G. Steven Bova
Prostate Cancer Research Center, Faculty of Medicine and Health Technology, Tampere University and Fimlab Laboratories Tampere, Finland

Background: The molecular changes necessary and sufficient for prostate cancer metastasis are poorly understood. Genomic studies including both primary and metastatic lesions from the same individual allow tracing of the genomic pattern associated with metastasis, and provide a dynamic context around which both basic science and clinical studies can shed light on means to improve cancer control.

Methods: In a pilot series of 33 men in various stages of completion, modifications of current pathology methods supported by new information technology. Whole genome, targeted, and transcriptome sequencing in blood and dissected tissue samples. Bioinformatic methods for tracing clonal evolution, identification of candidate truncal and non-truncal driver and passenger mutations, analysis of druggability of candidate driver mutations, and analysis of clinical timeline in relation tumor phylogeny.

Results/Discussion: Initial tracing of anatomic origins in the primary site suggest that only a minority of primary cancer cells contain the full set of mutations found in metastases. In the first case with integration of WGS and RNAseq data from multiple sites, WGS analysis revealed convergent evolution of independent AR gene amplification events in four metastases after initiation of androgen deprivation, and AR p.L702H mutation in four liver metastases after corticosteroid initiation. Transcriptome analysis revealed increased expression of AR-regulated genes in AR p.L702H mutant tumors, suggesting a dominant effect by a the mutation present in 1 of 16 copies in each cell. The metastases harbored several alterations to the PI3K/AKT pathway, including duplications of PIK3CA and AKT3, and mutations in PIK3CA and PIK3CG, the latter a truncal mutation. The list of truncal genomic alterations shared by all metastases also included homozygous deletion of TP53, hemizygous deletion of RB1 and CHD1, and amplification of FGFR1. If the patient were treated today, second-generation androgen-blockade, cessation of glucocorticoid administration, and therapeutic inhibition of the PI3K/AKT pathway or FGFR1 receptor could provide personalized benefit. Three previously unreported truncal clonal missense mutations were expressed at the RNA level and assessed as druggable. The truncal status of mutations may be critical for effective actionability and merits further study. These

and other recent results will be put in context of some recent studies from other laboratories.

Conclusion and Current Work: The findings suggest that a large set of deeply analyzed cases could serve as a powerful guide to more effective prostate cancer basic science and personalized cancer medicine clinical trials, and could better define the shoreline between primary, dormant metastatic and active metastatic disease.

Multi-organ mapping of cancer origins

Richard J. Gilbertson
Li Ka Shing Chair of Oncology, Head of Dept. of Oncology, Director, Cambridge Cancer Center, CRUK Cambridge Institute, Li Ka Shing Centre, Robinson Way Cambridge CB2 0RE, UK

Cancers are distributed unevenly across the body, but the importance of cell intrinsic factors such as stem cell function in determining organ cancer risk is unknown. This talk describes a study in which Cre-recombination of conditional lineage tracing, oncogene, and tumour suppressor alleles was used to define populations of stem and non-stem cells in mouse organs and test their life-long susceptibility to tumorigenesis. We show that tumour incidence is determined by the life-long generative capacity of mutated cells. This relationship held true in the presence of multiple genotypes and regardless of developmental stage, strongly supporting the notion that stem cells dictate organ cancer risk. Using the liver as a model system, we further show that damage-induced activation of stem cell function markedly increases cancer risk. Therefore, we propose that a combination of stem cell mutagenesis and extrinsic factors that enhance the proliferation of these cell populations, creates a "perfect storm" that ultimately determines organ cancer risk.

Inherited factors that influence cancer risk and behavior

Ian R. Tomlinson
Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham UK

For the common cancers, the numbers of predisposition genes that have been identified now number in the hundreds. By contrast, the identification of inherited factors that influence the management of cancer patients has been very slow, currently being limited to a handful of successes in pharmacogenetics and weak predictors of prognosis. Genetic studies of common cancer predisposition gene are, at last, starting to provide insights into disease biology. Using colorectal cancer as an exemplar, genetic studies have identified multiple factors that influence risk, not only including strong candidates such as Wnt signalling, but also "rediscovering" previously postulated variants, such as those at HLA loci. Other pathways, such as BMP signalling, have come to the fore as a result of these studies. However, there is also a large number of colorectal cancer risk genes with no clear link to tumorigenesis and these represent an attractive topic for further research. The set of predisposition genes has highlighted the fact that colorectal cancer risk factors generally act in normal tissues or cancer precursor lesions, and suggests that whilst mechanisms are heterogeneous, one major factor influencing colorectal cancer risk is variation in stem cell numbers or behaviour.

Insights from cancer genome sequencing: Utilising the totality of mutagenesis for clinical purposes

Serena Nik-Zainal
CRUK Advanced Clinician Scientist Department of Medical Genetics and MRC Cancer Unit The Clinical School, University of Cambridge, UK

A cancer genome carries the historic mutagenic activity that has occurred throughout the development of a tumour [1]. While driver mutations were the main focus of cancer research for a long time, passenger mutational signatures - the imprints of DNA damage and DNA repair processes that have been operative during tumorigenesis - are also biologically informative [1, 2]. In this lecture, I provide a synopsis of this concept, describe the insights that we have gained through combinations of computational analysis [3, 4] and experiments in cell-based systems [5], and showcase how we have developed the concept into applications that we hope to translate into clinical utility in the near future [3, 4]. I describe our efforts in a population-derived cohort as well as in individual patients, emphasizing the need for us to be more precise in analyses and interpretations in human cancer genomics.

1. Helleday T, Eshtad S, Nik-Zainal S. Mechanisms underlying mutational signatures in human cancers. Nat Rev Genet. 2014 Sep;15(9):585-98. doi: 10.1038/nrg3729. Epub 2014 Jul 1. Review. PubMed PMID: 24981601; PubMed Central PMCID: PMC6044419

2. Alexandrov LB, Nik-Zainal S, et al. Signatures of mutational processes in human cancer. Nature. 2013 Aug 22;500(7463):415-21. doi: 10.1038/nature12477. Epub 2013 Aug 14. Erratum in: Nature. 2013 Oct 10;502(7470):258. Imielinsk, Marcin [corrected to Imielinski,

Marcin]. PubMed PMID: 23945592; PubMed Central PMCID: PMC3776390

3. Nik-Zainal S, Davies H, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature. 2016 Jun 2;534(7605):47-54. doi: 10.1038/nature17676. Epub 2016 May 2. PubMed PMID: 27135926; PubMed Central PMCID: PMC4910866

4. Davies H, Glodzik D, et al. and Nik-Zainal S. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat Med. 2017 Apr;23(4):517-525. doi: 10.1038/nm.4292. Epub 2017 Mar 13. PubMed PMID: 28288110; PubMed Central PMCID: PMC5833945

5. Zou X, Owusu M, Harris R, Jackson SP, Loizou JI, Nik-Zainal S. Validating the concept of mutational signatures with isogenic cell models. Nat Commun. 2018 May 1;9(1):1744. doi: 10.1038/s41467-018-04052-8. PubMed PMID: 29717121; PubMed Central PMCID: PMC5931590

Mechanisms of Evasive Resistance in HCC

Michael N. Hall
Biozentrum, University of Basel, Switzerland

Cancer is a major health problem due to the failure of current therapies to effectively eradicate the disease. Extensive research over decades has led to the development of therapies that target cancer-specific signaling pathways. However, tumors escape such therapies by activating compensatory signaling pathways, a process referred to as 'evasive resistance'. The identities of the alternative signaling pathways and the functional interconnections that underlie evasive resistance remain widely unknown. We integrate clinical, molecular, and computational sciences to understand the signaling defects that enable tumors to evade therapy. Within the framework of rigorously designed clinical studies, hepatocellular carcinoma (HCC) tissue is isolated before therapy, during treatment, or at the time of tumor progression. The tumor tissue is obtained by needle biopsy and immediately snap frozen to preserve in vivo tumor properties. High- and low-throughput experimental and computational methods are then applied to determine the underlying signaling defects. This endeavor will elucidate mechanisms of evasive resistance and may ultimately improve cancer diagnosis, treatment and clinical outcome. Recent progress in this ambitious project and a related study with an mTOR-driven mouse model for HCC will be described.

Towards rational combinatorial cancer treatment – a functional genomics approach

Daniel S. Peeper
Netherlands Cancer Institute, Amsterdam, The Netherlands

For a long time, advanced-stage melanomas were refractory to the available therapeutic options, but developments in the current decade have begun to offer better perspectives for patients. The small molecule inhibitor vemurafenib, specifically targeting the mutant BRAFV600E kinase, was the first standard of personalized care for patients diagnosed with mutant BRAF metastatic melanoma. Although this compound initially reduces tumor burden dramatically, eventually most melanomas become resistant and progress on treatment. This occurs by the acquisition of additional mutations or other alterations, most of which reactivate the mitogen-activated protein kinase (MAPK) pathway. Although further suppression of BRAF-MAPK signaling by the inclusion of MEK inhibitor delays resistance, eventually most patients relapse.

The clinical outcome of late-stage melanoma patients has greatly improved also thanks to the recent availability of T cell checkpoint blockade, primarily by CTLA-4 and PD-1/PD-L1 antibodies. But still, large patient groups fail to (durably) benefit from these treatments, underscoring the continuing need for developing novel therapeutic modalities.

Therefore, in spite of these new clinical perspectives, there is a dire need to identify new targets amenable to therapeutic intervention, which ought to be applied in rational combination settings. We use function-based, genome-wide experimental strategies to develop rational combinatorial cancer treatment, targeting both cancer and immune cells. By screening for novel therapeutic targets and predictive biomarkers, we aim to achieve more durable clinical responses for patients. On the one hand, we are increasing our understanding of how cancer cells rewire their signaling networks, to expose and exploit new pharmacologically tractable tumor susceptibilities, also in the context of immunotherapy. On the other, we are manipulating various cell types from the patient's own immune system to boost their specific cytotoxicity towards tumor cells. With these approaches, we are developing new rational combinatorial therapies, which simultaneously eliminate the patients' tumor and harness their immune system.

Regulatory circuits in EMT, cell plasticity and cancer metastasis

N. Meyer-Schaller, D. Ronen, R. Kalathur, M. Saxena, D. Büchel, M. Diepenbruck, S. Pavan, A.N. Kilinc, S. Tiede, F. Lüönd, V. Vafaidazeh, and G. Christofori

Department of Biomedicine, University of Basel, Switzerland

A high degree of cell plasticity seems to promote malignant tumor progression, and an epithelial-mesenchymal transition (EMT) is suspected to provide cancer cells with increased cell plasticity for the development of metastasis and therapy resistance. Notably, EMT-induced cancer cell plasticity goes along with dedifferentiation processes, which are inherent to epithelial-mesenchymal plasticity. The distinct stages of an EMT are tightly regulated and involve activation and repression of a large number of genes that modulate the invasive behavior of cells and the formation of metastasis. Moreover, an increasing number of miRNAs and transcription factors reciprocally repress each other's expression during an EMT. Using genome-wide functional screen for kinases, phosphatases, transcription factors and miRNAs our laboratory has identified a number of novel signaling pathways and regulatory circuits underlying an EMT. Perturbation of these pathways reveals the epistatic hierarchy of the regulation of an EMT. Many of the signaling pathways and transcription factors critical for the regulation of an EMT are well known for their functions in the homeostasis of embryonic and somatic stem cells. We find that cells undergoing an EMT exhibit several hallmarks of stem cells and are more tumorigenic as compared to their epithelial counterparts. Most importantly, EMT-induced cancer cell plasticity can be therapeutically exploited by forcing the conversion of breast cancer cells into post-mitotic functional white adipocytes. This conversion is possible only in cancer cells that have undergone EMT and not in their epithelial ancestors and represses primary tumor invasion and metastasis formation in various in vivo models of breast cancer. The results indicate that acquired cell plasticity of invasive cancer cells is a major contributor to cancer invasiveness and metastasis. Thus, the identification of the genes and pathways enabling such cancer cell plasticity may open avenues to the development of innovative therapies.

Lactate exchange promotes oxidative stress resistance and melanoma metastasis

Alpaslan Tasdogan, Brandon Faubert, Vijayashree Ramesh, Ralph DeBerardinis, Sean Morrison

Howard Hughes Medical Institute and Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Metastasis is an inefficient process that requires cancer cells to undergo poorly-understood metabolic changes. Lactate has generally been considered a waste product that cancer cells must eliminate, but it was recently discovered that some cancer cells consume lactate and that this is correlated with worse outcomes in lung cancer. Here we show that lactate exchange promotes melanoma metastasis by reducing reactive oxygen species (ROS) and oxidative stress. Efficiently and inefficiently metastasizing patient-derived xenografts did not exhibit any difference in the uptake or use of isotopically labelled glutamine or glucose; however, efficient metastasizers took up more lactate and used it to fuel the TCA cycle. Efficient metastasizers expressed higher levels of Monocarboxylate Transporter 1 (MCT1) and MCT1 inhibition reduced lactate uptake in vivo. MCT1 inhibition had a limited effect on the growth of primary subcutaneous tumors but substantially reduced the frequency of circulating melanoma cells and metastatic disease burden. MCT1 inhibition significantly reduced flux through the pentose phosphate pathway and increased ROS levels. Treatment with the anti-oxidant N-acetylcysteine rescued the effects of MCT1 inhibition on ROS levels and metastasis. Melanoma cells thus become particularly dependent upon MCT1-mediated lactate exchange during metastasis to manage oxidative stress.

Codon-specific mRNA translation regulation in cancer

Pierre Close
Lab of Cancer Signaling, GIGA-Institute, University of LIEGE, Belgium

Translation reprogramming plays key roles in cancer development and drug resistance but the molecular mechanisms involved in this process remain poorly understood. Wobble tRNA modifications are required for specific codon decoding during mRNA translation. Our recent work highlighted surprising upregulation of the wobble uridine tRNA modification enzymes (U_{34}-enzymes) in several human cancers that underlies specific requirement of this pathway in tumor development (1) and progression to metastasis (2, 3). We hypothesized that such modifications may contribute to protein synthesis rewiring during oncogene-induced transformation and/or adaptation of cancer cells to therapy. Accordingly, we show that $BRAF^{V600E}$ melanoma cells are addicted to U_{34}-tRNA modification and that inhibiting the U_{34}-enzymes ELP3 and CTU1/2 synergizes with MAPK-targeting therapies in the killing of melanoma cells. Activation of PI3K/mTORC2-signaling pathway, the main mechanism of acquired resistance to MAPK-therapeutics, causes dramatic increase in U_{34}-enzymes levels. Mechanistically, U_{34}-enzymes promote melanoma cells glycolysis through a direct, codon-dependent, regulation of HIF1-alpha mRNA translation and the maintenance of high HIF1-alpha protein levels. Therefore, acquired resistance to anti-BRAF therapy is associated with high levels of U_{34}- enzymes and HIF1-alpha. These results demonstrate that U_{34}-enzymes promote melanoma cell survival and therapy resistance by regulating specific mRNA translation (4). Together, our work highlight the importance of codon-specific mRNA translation regulation in cancer.

1. A. Ladang et al., Elp3 drives Wnt-dependent tumor initiation and regeneration in the intestine. Journal of Experimental Medicine. 212, 2057–75 (2015).

2. S. Delaunay et al., Elp3 links tRNA modification to IRES-dependent translation of LEF1 to sustain metastasis in breast cancer. The Journal of experimental medicine. 213, 2503–2523 (2016).

3. F. Rapino, S. Delaunay, Z. Zhou, A. Chariot, P. Close, tRNA Modification: Is Cancer Having a Wobble? Trends in Cancer. 3, 249–252 (2017).

4. F. Rapino et al., Codon-specific translation reprogramming promotes resistance to targeted therapy. Nature. 558, 605–609 (2018).

Role of metabolism in supporting tumor progression

Matthew G. Vander Heiden
Koch Institute for Integrative Cancer Research at MIT, Cambridge MA 02139, USA

Complex regulatory mechanisms enable cell metabolism to match physiological state. The major pathways cells use to turn nutrients into energy and to synthesize macromolecules have been elucidated; however, there remain many unanswered questions regarding how metabolism supports cell proliferation and thus how best to target metabolism for cancer treatment. We have found that acquisition of some amino acids, including aspartate and serine, can be limiting for tumor growth. Both environmental and genetic factors that can influence how cells obtain critical nutrients and determining which nutrients are limiting for proliferation is one strategy to slow tumor growth. How genetic and tissue factors cooperate with extrinsic metabolic inputs to influence tumor progression will be discussed in the context of how best to exploit altered metabolism to treat cancer.

Dissecting tumor-genotype/immunophenotype relationships in metastatic breast cancer

Karin E. de Visser
Division of Tumor Biology & Immunology, Oncode Institute, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Metastasis formation is a key challenge in cancer patient care that urgently needs solutions. It is now well established that cells and mediators of the immune system influence these processes. Historically, our immune system has been considered to form an intrinsic defense mechanism against cancer and metastasis. Yet, the majority of cancer types exploit a myriad of strategies to successfully evade destruction by the immune system. In fact, mounting evidence supports the notion that cancer cells hijack the immune system for their own benefit, allowing them to escape from immune attack, maintain limitless proliferation, survive under dire circumstances and spread to distant organs. The overall goal of our research is to understand how the immune system influences metastasis formation. To achieve this, we utilize pre-clinical mouse models that faithfully recapitulate human breast tumorigenesis in combination with immune profiling studies in breast cancer patients. We have previously discovered that breast tumors elicit a systemic inflammatory cascade to dampen anti-tumor T cells and promote metastasis formation. Current efforts are underway to dissect how the genetic make-up of breast tumors dictates activation of systemic immunosuppressive inflammation. Our

findings provide novel mechanistic insights into the thus far poorly understood metastatic cascade, and open new avenues for the development of therapeutic strategies to unleash anti-tumor immunity and to inhibit metastatic disease.

Exploring and Exploiting the Tumour Microenvironment

Johanna A. Joyce
Ludwig Institute for Cancer Research, University of Lausanne, Switzerland

Cancers develop in complex tissue environments, which they depend upon for sustained growth, invasion and metastasis. Different tumour microenvironments (TME) are populated by diverse cell types including innate and adaptive immune cells, fibroblasts, blood and lymphatic vascular networks, and specialised organ-specific cell types, which collectively have critical functions in regulating tumorigenesis. We are interested in determining how reciprocal communication between cancer cells and diverse immune and stromal cell types in the TME regulates tumour initiation, progression, and metastasis, and additionally modulates the response to therapeutic intervention. Our latest findings will be presented, with a focus on our identification of new roles for innate immune cells in controlling these processes.

Cancer regulation by the bone marrow microenvironment

Paul Frenette
Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, New York, USA

Hematopoietic stem cells (HSCs) have the ability to maintain hematopoietic output throughout an organism's lifespan. Upon aging, HSCs suffer a decline in their regenerative capacity and multi-lineage differentiation potential, leading to impaired immune responses and the development of hematologic malignancies. We have recently found that aging is associated with significant reductions in innervating adrenergic nerve fibers in the bone marrow. Denervation of the bone marrow of young mice produces all the hallmarks of HSC aging, including myeloid bias with lymphopenia, loss of polarization, and diminished repopulation capacity. Supplementation of a β3-adrenoreceptor agonist to old mice significantly rejuvenated the in vivo function of aged HSCs by acting on bone marrow stromal cells, suggesting that the preservation or restitution of marrow SNS innervation may hold the potential for new HSC rejuvenation strategies. Among prevalent hematologic malignancies of aged individual, acute myeloid leukemia (AML) has a poor outcome. Our recent studies investigating the role of the macrophage niche in hematopoiesis have revealed novel functions for Vascular Cell Adhesion Molecule 1 (Vcam1), an adhesion molecule known to be expressed on macrophage, endothelial and stromal cells, where to mediate homing and retention of HSCs and progenitors into bone marrow. We found that Vcam1 was also expressed on HSCs, and upregulated in human (and mouse) AML. High Vcam1 expression was associated shorter survival. We will present data suggesting that Vcam1 plays a role in the recognition of "self", regulating AML progression via clearance inhibition by phagocytosis. Targeting VCAM1 may represent a promising approach to eliminate leukemic stem cells in AML.

Overcoming resistance to PD-1 blockade therapy

Antoni Ribas
University of California Los Angeles (UCLA), USA

The main reason for primary resistance to PD-1 blockade, defined as lack of response to upfront therapy, is the absence of a pre-existing intratumoral tumor antigen-specific T cell infiltration (1). In a minority of these cases with primary resistance, there may be a genetic mechanism of primary resistance to PD-1 blockade therapy that is a result of strong immunoediting of the cancer genome under continuous pressure by the immune system. This is particularly evident in cancers with a high mutational load (high antigenicity), resulting in the genetic inactivation of key molecules in the antigen presentation pathway (beta-2 microglobulin – B2M- or HLAs) or in the interferon gamma signaling pathway (JAK1 or JAK2) (2, 3). Approximately one third of patients with metastatic melanoma who initially had an objective tumor response to PD-1 or CTLA-4 blockade therapy develop acquired resistance, with a delayed tumor relapse. Mechanisms are clearly poorly understood (4). In some instances, acquired resistance is also induced by a genetic immunoediting mechanism resulting in loss of function mutations in the interferon gamma receptor or the antigen presentation pathway (5). Work in the clinic to overcome resistance has focused on combination immunotherapies. The intratumoral injection of oncolytic viruses or TLR agonists can trigger a local type I interferon response, given together with anti-PD-1 therapy to result in a systemic response. Early clinical trials with such combinations suggest a higher response upfront with objective responses in patients without a pre-existing T cell infiltrate, as well as responses in patients who had previously progressed on prior anti-PD-1 therapy. Furthermore, there is increasing evidence that the combination of PD-1 blockade with targeted therapies with BRAF and MEK inhibitors is safe and can result in an increased frequency of long lasting tumor responses. These and other combinations can increase response rates or reverse resistance to anti-PD-1 therapy (4).

1. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515:568-71.

2. Grasso CS, Giannakis M, Wells DK, Hamada T, Mu XJ, Quist M, Nowak JA, Nishihara R, Qian ZR, Inamura K, Morikawa T, Nosho K, Abril-Rodriguez G, Connolly C, Escuin-Ordinas H, Geybels MS, Grady WM, Hsu L, Hu-Lieskovan S, Huyghe JR, Kim YJ, Krystofinski P, Leiserson MDM, Montoya DJ, Nadel BB, Pellegrini M, Pritchard CC, Puig-Saus C, Quist EH, Raphael BJ, Salipante SJ, Shin DS, Shinbrot E, Shirts B, Shukla S, Stanford JL, Sun W, Tsoi J, Upfill-Brown A, Wheeler DA, Wu CJ, Yu M, Zaidi SH, Zaretsky JM, Gabriel SB, Lander ES, Garraway LA, Hudson TJ, Fuchs CS, Ribas A, Ogino S, Peters U. Genetic Mechanisms of Immune Evasion in Colorectal Cancer. Cancer Discov. 2018;8:730-49.

3. Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Hu-Lieskovan S, Kalbasi A, Grasso CS, Hugo W, Sandoval S, Torrejon DY, Palaskas N, Rodriguez GA, Parisi G, Azhdam A, Chmielowski B, Cherry G, Seja E, Berent-Maoz B, Shintaku IP, Le DT, Pardoll DM, Diaz LA, Jr., Tumeh PC, Graeber TG, Lo RS, Comin-Anduix B, Ribas A. Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations. Cancer Discov. 2017;7:188-201.

4. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell. 2017;168:707-23.

5. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, Torrejon DY, Abril-Rodriguez G, Sandoval S, Barthly L, Saco J, Homet Moreno B, Mezzadra R, Chmielowski B, Ruchalski K, Shintaku IP, Sanchez PJ, Puig-Saus C, Cherry G, Seja E, Kong X, Pang J, Berent-Maoz B, Comin-Anduix B, Graeber TG, Tumeh PC, Schumacher TN, Lo RS, Ribas A. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. N Engl J Med. 2016;375:819-29.

Targeting BCL2 as therapy for haematological malignancies: from concept to emerging clinical applications

Andrew W. Roberts (1, 2, 3, 4)
1. Cancer and Haematology Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia, 2. Clinical Haematology, The Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Parkville, 3. Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, 4. Victorian Comprehensive Cancer Centre, Parkville, Australia

In order to survive the major stresses that oncogenic mutations impose, many haematological malignancies harbour aberrations that circumvent apoptosis. Dysfunction of the TP53 pathway and dysregulation of the BCL2 family are the most common. The development of a new class of small molecule anti-cancer drugs, called BH3-mimetics, means that we now have therapies that can directly trigger apoptosis in cells that are dependent on highly expressed prosurvival proteins, such as BCL2, MCL1 and BCLxL. The focus of this presentation is on the development of the selective BCL2 inhibitor, venetoclax, and its first approved clinical indications. Through this prism, general lessons about the potential and pitfalls of using BH3 mimetics will be considered.

As a single agent, venetoclax is highly active in vitro and in clinical trials against chemotherapy-refractory chronic lymphocytic leukaemia (CLL), mantle cell lymphoma and acute myeloid leukaemia (AML), and has some activity against myeloma and some other lymphomas. In early phase clinical trials, venetoclax induced objective responses in approximately 80% of patients with relapsed or refractory CLL, including complete remissions in 20%. Similar results were observed in previously treated mantle cell lymphoma. In these diseases, depth of response was correlated with durability of response. Venetoclax kills cells in a TP53-independent fashion. Consistent with this, it proved highly effective as monotherapy in patients with relapsed CLL bearing deletion of the long arm of chromosome 17, and was approved by the FDA and EMA in 2016 for this indication. The risk of relapse however is high, especially in patients with complex karyotype or who have had many lines of previous therapy. Recent insights to a variety of clinically relevant mechanisms of resistance will be explored. Finally, early clinical trial evidence indicates that higher response rates and more durable responses can be achieved using rational combinations of venetoclax with monoclonal antibodies, tyrosine kinase inhibitors and hypomethylating agents in CLL, lymphoma and AML respectively. These and other combinations currently studied preclinically will be reviewed to try and provide a sense of how venetoclax may be best used in the future.

Curing CLL: A glimpse into the future of cancer treatment?

Peter Hillmen
Professor of Experimental Haematology, University of Leeds, UK

Chronic lymphocytic leukaemia (CLL) is the most common leukaemia occurring in approximately 6 per 100,000 of the population and is currently incurable for the large majority of patients. CLL is almost unique in that as a blood and marrow based disease frequent sampling of the tumour during and after therapy is easily done providing a good model for understanding the development of genomic complexity and resistance to therapy at multiple time-points throughout treatment. Regardless of the therapy used the eradication of CLL to below detectable levels (minimal residual disease [MRD] negative) is associated with improved outcomes and is a prerequisite for cure. CLL is characterised in all cases by the disruption of two key B-cell pathways – chronic proliferation mediated through the B-cell receptor signalling and the inhibition of apoptosis due to the upregulation of B-cell lymphoma-2 (Bcl-2). The relatively recent development of targeted therapy to these pathways, with inhibition of Bruton's tyrosine kinase (Btk) with ibrutinib and the antagonism of Bcl-2 with venetoclax, has resulted in significant improvements in outcome for patients with CLL. In addition, monoclonal antibodies to CD20, particularly obinutuzumab, are effective when combined with chemotherapy or venetoclax in CLL. However it is clear that subsequent genetic evolution, particularly under the selection of therapy, potentially leads to resistance to therapy whether this be conventional cytotoxic chemotherapy or targeted therapies. However recent studies indicate that the cure of CLL is realistic and will be achieved by the application of combined targeted therapies as the initial therapy before genomic complexity occurs through the selective pressure of therapy. Various combinations of targeted therapies in CLL in patients with relapsed or refractory disease lead to a high proportion of patients achieving MRD negativity whereas the early data with these combinations in previously untreated patients suggests that the vast majority of patients will experience MRD negative remissions. The demonstration that a high proportion of patients with CLL experience eradication of MRD indicates that this approach will lead to prolonged remissions, the cessation of therapy, reduce the risk of resistance and is a curative approach. The use of novel agents in combination, disease modelling demonstrating that MRD eradication will improve outcomes, the move to cure and development of adaptive trials to demonstrate this will be discussed.

1. Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus Ofatumumab in Previously Treated Chronic Lymphoid Leukemia. N Engl J Med, 2014, Jul 17; 371(3):213-23.

2. Burger JA, Tedeschi A, Barr PM,et al. Ibrutinib as Initial Therapy for Patients with Chronic Lymphocytic Leukemia. N Engl J Med. 2015 Dec 17;373(25):2425-37.

3. Kwok M, Rawstron AC, Varghese A, et al. Minimal residual disease is an independent predictor for 10-year survival in CLL. Blood. 2016 Dec 15;128(24):2770-2773. Epub 2016 Oct 3. PMID: 27697770

4. Landau DA, Sun C, Rosebrock D, et al. The evolutionary landscape of chronic lymphocytic leukemia treated with ibrutinib targeted therapy. Nat Commun. 2017 Dec 19;8(1):2185.

5. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. N Engl J Med. 2018 Mar 22;378(12):1107-1120.

Highly multiplexed imaging of tissues with subcellular resolution by imaging mass cytometry

Bernd Bodenmiller
University of Zurich, Institute of Molecular Life Sciences, Zurich, Switzerland

Heterogeneous cancer cells and stromal and immune cells form a dynamic ecosystem that evolves to support tumor expansion and ultimately tumor spread. The complexity of this dynamic ecosystem is a main obstacle in our attempts to treat and heal cancer. The study of tumor ecosystems, including the cells and their communication, is thus essential to enable an understanding of tumor biology, to define new biomarkers to improve patient care, and ultimately to identify new therapeutic routs and targets.

To study and understand the workings of tumor ecosystems, highly multiplexed image information of tumor tissues is essential. Such multiplexed images will reveal which cell types are present in a tumor, their functional state, and which cell-cell interactions are present. To enable multiplexed tissue imaging, we developed imaging mass cytometry (IMC). IMC uses metal isotopes of defined mass as reporters and currently allows to visualize over 50 proteins and transcripts simultaneously on tissues with subcellular resolution. We applied imaging mass cytometry to samples from 281 breast cancer patients for whom long-term survival and treatment data was available and created high-dimension immunohistochemistry pathology images. Single-cell segmentation and analysis quantified tumor and stromal single-cell phenotypes, their interactions, and spatial heterogeneity. Classification based on multi-dimensional cellular composition and tissue organization categorized the cellular structure of the breast cancer tumor microenvironment, and identified novel breast cancer subtypes with distinct clinical prognosis and response to therapy.

These findings show that highly multiplexed single-cell pathology can stratify cancers with distinct clinical outcomes and suggests cellular targets for patient-specific therapeutic intervention.

Patient-derived organoids: promises, hurdles and potential clinical applications

Nicola Valeri
ICR The Institute of Cancer Research, Sutton, UK

The limited role of genomic profiling in predicting response to targeted therapies and limitations of pre-clinical models currently used for drug validation represent important obstacles hampering the success of personalized medicine and drug discovery.
In vivo functional genomics has been proposed as a way to overcome some of the hurdles in understanding the molecular complexity of drug response and resistance. Co-clinical trials matching drug response in patients and related pre-clinical models represent a promising strategy to personalize treatment and
understand mechanisms of chemo-sensitivity through reverse translation. Most co-clinical trials rely on the use of genetically engineered mouse models or patient-derived xenografts. Although these classes of animal models can sometimes closely mirror clinical scenarios, their use poses several logistic, ethical, and economic issues. Thus, there is an unmet need to develop robust, rapid, and cost-effective pre-clinical models of metastatic cancers.
LGR5+ stem cells can be isolated from a number of organs and propagated as epithelial organoids in vitro. Mouse and human organoids have been used to study the physiology and neoplastic transformation of the liver, pancreas, bowel and prostate among other organs.
Patient derived organoids (PDOs) were established from ultrasound and/or CT scan guided tissue biopsies from metastatic, chemo-refractory, heavily pre-treated colorectal and gastroesophageal cancer patients recruited in phase I/II clinical trials. Sequential (pre- and post-treatment PDOs were obtained in a number of patients). Genomic and transcriptomic profiling of PDOs were compared to the ones of their parental biopsies and that of the primary cancer where available. High-throughput drug screening using a library of compounds in early clinical trials or clinical practice was used for 3D drug testing.
Phenotypic, genotypic and transcriptomic profiling of PDOs was compared to their matching tumour demonstrating high similarity between the two. Drug screening results were matched with the molecular profiling of PDOs in analysing response to targeted agents or drug combinations, supporting the notion that PDOs could complement sequencing approaches in defining cancer vulnerabilities.
Ex vivo responses to anticancer agents in PDOs and PDO-based orthotopic xenograft tumour mouse models were compared head-to-head with response observed in their respective patients in the context of clinical trials showing that PDOs have 100% negative and 88% positive predictive power in forecasting treatment responses. Our data suggest that PDOs are capable of recapitulating responses observed in the clinic, and have the potential to be implemented in precision medicine programs.

Poster Abstracts

1

Harnessing lymphoid neogenesis for prognosis and treatment of solid tumours

K. Silina (1), A. Soltermann (2), F. Movahedian Attar (1), R. Casanova (2), A. Curioni-Fontecedro (4), H. Moch (2), F. Posch (5), T. Winder (2), N. Van Dijk (3), C. Voskuilen (3), M. Van der Heijden (3), M. Van den Broek (1)

(1) Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland, (2) Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland, (3) Division of Medicine, The Netherlands Cancer Institute, Amsterdam, The Netherlands, (4) Center for Haematology and Oncology, University Hospital Zurich, Zurich, Switzerland, (5) Department of Oncology, Medical University Graz, Graz, Austria

Lymphoid organ neogenesis takes place in chronically inflamed tissues including cancer and yields the development of tertiary lymphoid structures (TLS). TLS are ectopic lymphoid organs that activate antigen specific T cells and B cells in infection and autoimmunity and correlate with prolonged survival in various cancer types. This suggests that TLS contribute to protective anti-tumor immunity. Therefore, promoting the development of tumor-associated TLS could be a novel immunotherapeutic approach. However, the molecular and cellular mechanisms of TLS development in human cancer or how TLS contribute to survival are largely not understood.

Here we used multiparameter immunofluorescence and digital pathology to quantify TLS and to characterize their cellular composition and tissue context in cohorts of lung squamous cell carcinoma (LSCC, n=138), colorectal cancer (CRC, n=111), clear cell renal cell carcinoma (ccRCC, n=50) and bladder cancer (BC, n=33) patients. Furthermore, we established an experimental model to characterize TLS development and its impact on tumor-specific immunity.

We discovered that TLS development and maturation followed the same steps in all analyzed tumor types as well as in the lungs of mice in our experimental model. First, B and T lymphocytes accumulated around blood vessels. Second, a network of follicular dendritic cells developed within the lymphocytic aggregate and third, a germinal center (GC) reaction was activated. We found that the number of tumor-associated TLS was an independent prognostic factor for prolonged survival in untreated LSCC, CRC and BC, but not in ccRCC patients or in LSCC and BC patients that were treated with neoadjuvant chemotherapy. By comparing the chemotherapy-treated and untreated cohorts we observed that the number of TLS was not changed but TLS maturation (i.e. GC formation) was significantly impaired after chemotherapy. This difference was at least partially dictated by corticosteroids, which are commonly used to treat the side-effects of chemotherapy of LSCC patients.

We further studied the mechanisms underlying TLS development using the experimental model. We identified a combination of stimuli that induces the development of mature TLS in the lungs of mice. Besides inflammatory stimuli, a foreign antigen was necessary to achieve a significant increase in TLS numbers and maturation stage, suggesting that cognate interactions are crucial for lymphoid organ neogenesis. This is further supported by our observation that CRC patients with microsatellite instability, which presumably results in more neo-antigens, had an increased proportion of mature TLS. The negative impact of corticosteroids on TLS development was confirmed in this model.

In summary, we propose that GC+ TLS represent the relevant TLS phenotype contributing to survival in different tumor types. Lymphoid organ neogenesis is negatively affected by corticosteroids, which might impair the spontaneous as well as therapy-induced anti-tumor immunity. The established experimental model will allow investigation of the mechanisms of TLS development and function in cancer and to assess their therapeutic potential.

2

Colon cancer infiltrating neutrophils as potential target to improve anti-tumor T-cell responses

A M. Germann (1), AD. Bowler (1), M. O. Sauvain (4), C. Sempoux (3), G. Coukos (2), F. Radtke (1)

(1) ISREC, EPFL, Lausanne, (2) Department of Oncology, CHUV, Lausanne, (3) Department of Pathology, CHUV, Lausanne, (4) Department of Visceral Surgery & Department of Oncology, CHUV, Lausanne

It is well established that in cancer patients the capability of the immune system to raise an immune response against tumor cells is essential to counteract disease progression. In colon cancer recent studies have demonstrated that infiltration of primary tumors with CD8+ cytotoxic T-cells (CTLs) is associated with good disease outcome in human patients. Similarly, presence of CTLs at the margin of colon cancer metastasis associates with patient response to adjuvant chemotherapy after resection of primary tumors. Therefore, methods to boost a CTL response against cancer cells have a high therapeutic potential for colon cancer patients. Here we demonstrate that human

and mouse colon tumors are heavily infiltrated by neutrophils with a strong T-cell suppressive phenotype.
In a genetically engineered mouse model, T-cells counteracted colon tumorigenesis, but were lost during disease progression. This correlated with a substantial infiltration of neutrophils into tumors. When isolated, neutrophils showed an immune suppressive gene expression signature and efficiently suppressed T-cell proliferation in vitro. Neutrophil depletion in tumor bearing mice increased tumor T-cell activation and reduced tumor size. In human colon cancers, the majority of investigated tumors displayed high neutrophil infiltration. Neutrophils were recurrently juxtaposed to CTLs at the tumor margin, indicating that these cells could influence an anti-tumor T-cell response also in human disease. Taken together our results suggest that in colon cancer neutrophils could be substantially involved in the suppression of anti-tumor T-cell responses and could represent a valuable target for new therapeutic approaches.

3

Transcriptomic profiling of the Glioblastoma-BBB - towards molecular characterization and improved GBM therapy

J. B. Schaffenrath, M. Neidert, L. Regli, A. Keller

Department of Neurosurgery, University Hospital Zurich, University of Zurich, Zurich CH8091, Switzerland

Glioblastoma multiforme (GBM) is the most common primary brain tumor with high severity and low therapeutic success. GBM affects mostly aged individuals. Due to demographic changes towards an aging population, the GBM rate is predicted to increase. However, currently available therapies are not effective and the life expectancy remains in average 12-15 month after diagnosis.
One important aspect in treating GBM is to achieve an effective drug concentration in tumor. It is well known that GBM vasculature is leaky to plasma proteins; however, the drug delivery into the GBM is not successful. Most of prescribed drugs are small lipid soluble molecules, which are effectively removed from the brain endothelial cells via ABC transporters. Until now, the development and homeostatic regulation of human blood-brain barrier (BBB) is poorly understood. The BBB is a multicomponent system and extending the knowledge about the molecular composition of the GBM BBB will hopefully bring insights into biology of GBM vasculature, and help to design better treatment strategies.
We aim to characterize the transcriptome of the human BBB in health and GBM. Therefore, endothelial cells (EC) are isolated from GBM and normal brain tissue using anti-human CD31 antibody coupled to magnetic beads, followed by lysis and isolation of RNA, which is subsequently subjected to RNA sequencing.
Our preliminary analysis of RNA sequencing data shows deregulated expression of SLC transporters in GBM endothelial cells compared to controls, whereas the expression of several ABC transporters remained unaltered. Preliminary analysis suggests that deregulation of several transporters (e.g. SLC2A1) in GBM ECs maybe occurring due to loss of BBB-specific phenotype of GBM EC. Furthermore, we want to understand which BBB characteristics (i.e. closed cell-cell junctions, expression of SLC and ABC transporters, low rate of transcytosis) are specifically altered in GBM ECs and whether we will find GBM specific changes that can be exploited for drug delivery (e.g. upregulation of a specific SLC transporter).

4

Immune profile based risk score for patients with malignant pleural effusion

C. G. Wu (1), F. Mairinger (2), A. Soltermann (1)

(1) Institute of Pathology and Molecular Pathology, University Hospital Zurich and University of Zurich, Zurich, Switzerland
(2) Institute of Pathology, University Hospital Essen, Essen, Germany

Background: Malignant pleural effusions (MPE) is a common clinical problem with no effective therapy, in which lung adenocarcinoma was with poor prognosis and highest incidence. Diverse immune cells and immune checkpoints such as PD-1 and its ligand (PD-L1) play important parts in tumor-associated microenvironment. However, their prognostic roles have not yet been well investigated in MPE.
Patient and methods: Cores from MPE cell blocks of 158 patients were assembled together on hybrid cytology-tissue microarrays (C/TMA). Three different PD-L1 antibody colons (SP263, E1L3N, Quartett) for IHC staining were processed. Expression analysis of MPO, CD3, CD4, CD8, CD20 and CD68 were performed by immunohistochemistry (IHC) and scored by pathologists and quantitatively counted using digitalized imaging analysis. 75 lung adenocarcinoma were profiled for immune gene expression for 770 genes and results were validated in a 40 gene panel with 88 MPE and matched primary lung adenocarcinoma samples by NanoSting analysis. Data were correlated with clinic-pathologic and clinical chemistry parameters.

Results and Conclusions:

- MPE patients with Lung or melanoma or GIT AdenoCa have dismal prognosis.
- Heterogeneities of tumour immune-microenvironment not only existed between cancer types but also within the same type between patients.
- PD-L1 (50% cutoff), CD3+ cells, neutrophils to CD8+ cells ratio and neutrophils to CD68+ cells ratio were potential prognostic-factors in MPE.
- In MPE Lung AC, PD-L1 (50% cutoff), TTF-1, CD4+ cells, Neutrophils, Neutrophils to CD3+, CD4+, CD8+, CD68+ ratios were prognostic parameters.
- Patients with higher expression of adaptive system activation and cytotoxic immune cells relevant genes had better survival.
- In MPE Lung AC, PD-L1 high and low expression patients had different immune-microenvironment profiling; Exhausted CD8 vs. TILs were significantly higher in PD-L1 high expression group.
- Our risk score system may predict survival for MPE Lung AC patients using transcriptomics data.

5

Towards the development of inhibitors targeting DNA repair pathways

A. Trenner, A.A. Sartori

Institute of Molecular Cancer Research, University Zurich, Zurich

Efficient DNA repair mechanisms are pivotal to counteract genomic instability, a hallmark of almost all cancer cells. DNA double-strand breaks (DSBs) are the most hazardous lesions a cell can encounter as a single unrepaired DSB leads to cell death, whereas erroneous repair results in mutations, which in turn can promote carcinogenesis. On the other hand, conventional cancer treatment by radiotherapy and certain chemotherapeutic drugs takes advantage of the cytotoxic properties of DSBs. However, these agents often lack selectivity for tumor cells, resulting in severe side effects for the patients, thus compromising their therapeutic potential. Hence, new strategies are key to the development of novel compounds that display synergistic effects with standard anti-cancer drugs by specifically targeting DSB repair mechanisms. In particular, homologous recombination (HR) as a repair mechanism for DSBs is indispensable for cancer cell survival. Importantly, the function of BRCA2 and CtIP, two critical HR factors, strongly relies on protein-protein interactions (PPIs). Consequently, specific targeting of these PPI interfaces could significantly improve the efficacy of conventional anti-cancer therapies. However, inhibition of PPIs has proven challenging as PPI interfaces commonly do not support binding of small drug-like molecules. In contrast, peptide-based inhibitors of PPIs are considered more promising but their therapeutic use is frequently limited by conformational and proteolytic instability as well as cell membrane impermeability. Importantly, artificial backbone modifications and the use of cell-penetrating peptides (CPPs) as delivery vectors were shown to significantly improve pharmaceutical properties of peptides.
In this project, we apply those strategies in order to design potent peptide-based HR inhibitors targeting BRCA2 and CtIP, opening up new therapeutic avenues to combat cancer.

6

FAP-4-1BBL: A Novel versatile tumor-stroma targeted 4-1BB agonist for combination immunotherapy with checkpoint inhibitors, T cell bispecific antibodies and ADCC-mediating antibodies

C. Claus, J. Sam, C. Ferrara-Koller, V. Nicolini, S. Colombetti, M. Bacac, P. Umana, C. Klein

Roche Innovation Center Zurich

For tumor specific co-stimulation via 4-1BB (CD137) a novel tumor-stroma targeted FAP-4-1BB ligand (FAP-4-1BBL, RG7827) was designed composed of a trimeric split 4-1BBL, a tumor-stroma targeted 4B9 Fab moiety recognizing fibroblast activation protein (FAP) and a heterodimeric Fc-region devoid of FcgR binding but maintained FcRn binding. In presence of a TCR signal 1 either from a T cell receptor MHCI/peptide interaction or through TCR engagement by a T-cell bispecific antibody, FAP-4-1BBL provides co-stimulation to T cells strictly dependent on cross-linking by FAP-expressing fibroblasts. Similarly, FAP-4-1BBL can provide co-stimulation to NK cells with activated FcgRIIIa signaling. FAP-4-1BBL was tested as monotherapy and combined with CEA-TCB, an anti-CEA T-cell bispecific antibody, in the s.c. gastric MKN45 xenograft model co-grafted with NIH-3T3 fibroblasts in human stem cell engrafted (HSC) NSG mice in comparison to DP47-4-1BBL, an analogous untargeted 4-1BBL fusion protein. For use in syngeneic mouse models in immunocompetent mice muFAP-4-1BB, a murine surrogate made of a mu4-1BB agonistic surrogate antibody fused to the variable region of the FAP antibody 28H1, was used. muFAP-4-1BB was tested as monotherapy and combined with a murine specific CEA-TCB surrogate (muCEA-TCB) and the muPD-L1 specific

surrogate antibody 6E11 in the s.c. colorectal MC38-CEA model in CEA transgenic (Tg) C57BL/6 mice. For combination with ADCC-mediating antibodies FAP-mu4-1BBL, a hybrid FAP-mu4-1BBL fusion protein, was generated. FAP-mu4-1BBL was tested as monotherapy and combined with the ADCC-mediating anti-HER2 antibody trastuzumab in the s.c. gastric N87 xenograft model in human CD16 Tg Scid mice. In the s.c. gastric MKN45 xenograft model co-grafted with NIH-3T3 fibroblasts in HSC-NSG mice FAP-4-1BBL resulted in combined anti-tumoral efficacy in combination with CEA-TCB, whereas the respective monotherapies as well as the combination with the untargeted DP47-4-1BBL did not improve anti-tumor efficacy. In the syngeneic s.c. MC38-CEA model in CEA Tg C57BL/6 mice, a model with natural FAP expression due to fibroblast infiltration, the muFAP-4-1BB surrogate antibody resulted in combined anti-tumor efficacy, both combined with muCEA-TCB and muPD-L1 antibodies including the induction of tumor remission. Finally, the hybrid FAP-mu4-1BBL surrogate resulted in improved anti-tumor efficacy combined with the ADCC-mediating antibody trastuzumab in the s.c. gastric N87 xenograft model in human CD16 Tg Scid mice. These data show that FAP-4-1BBL is a versatile combination partner for cancer immunotherapy mediating FAP-dependent co-stimulation to T and NK cells in combination with PD-L1 checkpoint inhibition, TCBs and ADCC-mediating antibodies. Clinical evaluation of this novel therapeutic approach is currently ongoing.

7

Tracing Epithelial-to-Mesenchymal Transition in Breast Cancer

F. Lüönd, N. Sugiyama, R. Bill, T. Lopes, T. Bürglin, C. Hager, G. Christofori

Department of Biomedicine, University of Basel

Epithelial-to-mesenchymal transition (EMT) is a gradual process of dedifferentiation, which conveys carcinoma cells with enhanced migratory and invasive capabilities, stem cell-like properties and drug resistance. Due to the transient and reversible nature of the process, the extent to which cancer cells may undergo a spontaneous EMT in vivo remains obscure.

We have developed two genetic lineage tracing systems to visualize cancer cells that undergo an EMT in a mouse model of metastatic breast cancer. Based on the expression of an early- or late-stage EMT-marker, cancer cells in a “partially” epithelial/mesenchymal or “fully” mesenchymal state switch from mCherry to GFP expression. In the early-stage EMT tracing model, a low number of GFP positive cells is observed. These cells are either found as isolated E-cadherin negative, spindle shaped cells at the tumor-stroma interface or within the stroma, as E-cadherin low, elongated cells within stromal-rich areas or as E-cadherin high populations in epithelial lesions, indicating that these cells, after having undergone an EMT, re-differentiate and perform a mesenchymal-to-epithelial transition. In contrast, extremely few GFP positive cells are found in the late-stage EMT tracing model. These cells mostly retain an E-cadherin negative, spindle-shaped mesenchymal phenotype and are localized within the tumor stroma.

In conclusion, we have developed two lineage tracing systems to study the contribution of partial and full EMT towards distinct aspects of breast cancer malignancy. Our observations suggest that cancer cells mostly transition between epithelial/mesenchymal hybrid-stages but rarely complete a full EMT.

8

New insights into PDGF/PDGFR axis in renal cell carcinomas

A. M. Cimpean (2), A. D. Cumpanas (2), A. Cumpanas (2), A. Nesiu (1), I. Ioiart (1), M. Raica (2)

(1) Department Of Urology, Vasile Goldis University, Faculty Of Medicine, Arad, Romania, (2) Department Of Microscopic Morphology/Histology, Angiogenesis Research Center, Victor Babes University Of Medicine And Pharmacy Timisoara, Romania

Patients with clear cell renal cell carcinoma (CCRCC) have a highly heterogeneous response to therapy and resistance to therapy appears early during treatment. We aim to study PDGF pathway expression profile correlated with tumor vessels type, grade invasion and growth factors expression. Fifty cases of CCRCC were preliminary evaluated by histopathology and selected for immunohistochemistry and molecular analysis. Cases evaluation included histopathology, PDGF assessement by immunohistochemistry and RNAscope, and tumor grouping with emphasis to tumor vessels types previously described by our team. For molecular analysis we used TaqMan array for PDGF pathway applied to PDGF Pathway 96-well plates containing 92 genes and 4 control genes. Results were evaluated by DATA ASSIST software and gene expression profile was correlated with grade, invasion, tumor vessels types, VEGF. All CCRCC were positive for PDGF BB by immunohistochemistry and RNAscope and 91,6% out of these cases being confirmed by RT PCR. Difference in gene expression profile were observed when we grouped cases according with tumor vessels types. PIK3C3 and SLC9A3 were significantly correlated with reticular and diffuse pattern of tumor vessels types but 5 different genes (STAT1, JAK2, SHC2, SRF and CHUK) were exclusively overexpressed in diffuse pattern compared with reticular pattern of blood vessels. Also, when we grouped cases according with tumor grade, SLC9A3, CHUK and STAT3 were overexpressed in G3 compared with G2. No significant correlation has been found between PDGF gene expression and invasion. PIK3C3 and SLC9A3 were differentially overexpressed according with intensity of VEGF. We may conclude that CCRCC showed a high molecular heterogeneity of PDGF pathway gene expression profile with an impact on early maturation of tumor vessels previously observed. Also, this heterogeneous gene expression profile may explain the different response to targeted therapies and in part resistance to therapy differently developed amongst patients.

9

Neutrophils Bind to Circulating Tumor Cells to Promote Cell Cycle Progression in the Bloodstream

B. Szczerba (2), F. Castro-Giner (2), M. Vetter (3), S. Gkountela (2), J. Landin (3), I. Krol (2), M. Scheidmann (2), C. Donato (2), R. Scherrer (2), J. Singer (1), C. Beisel (1), C. Kurzeder (3), WP. Weber (3), C. Rochlitz (3), V. Heinzelmann-Schwarz (3), N. Beerenwinkel (1), N. Aceto (2)

(1) Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland, (2) Department of Biomedicine, University of Basel, Switzerland, (3) University Hospital Basel, Switzerland

Circulating tumor cells (CTCs) are precursors of metastasis in several cancer types, but their biological features and vulnerabilities are poorly characterized. For instance, CTCs can be occasionally found in the bloodstream of patients as CTC-white blood cell (WBC) aggregates, yet, the role of these CTC-WBC clusters in the metastatic process is largely unknown. Here, we interrogate the identity and function of these CTC-associated WBCs, as well as the molecular features that define the interaction between tumor and immune cells in circulation. Using a combination of microfluidic devices and micromanipulation technologies, we isolated individual CTC-associated WBCs, in parallel with corresponding cancer cells within each CTC-WBC cluster. Single-cell RNA profiling of CTC-associated WBCs reveals a myeloid-like expression profile, indicating neutrophils as the most frequent type of CTC-associated WBCs. Further, the presence of neutrophils strongly promotes cell cycle progression of CTCs, and disruption of CTC-neutrophil clusters substantially impairs metastasis formation. Thus, we identify CTC-neutrophil clusters as previously unappreciated, highly efficient metastatic precursors in breast cancer, providing a rationale for targeting CTC-WBC interactions in breast cancer.

10

Oncogenic signaling of FGFR2 relies on truncation of its C-terminal tail

D. Zingg (1), SM. Kas (1), S. Annunziato (1), B. Bhin (1), JR. De Ruiter (1), C. Lutz (1), E. Schut (1), S. Klarenbeek (3), E. Wientjens (1), M. Van Miltenburg (1), A-P. Drenth (1), LFA. Wessels (2), J. Jonkers (1)

(1) Division of Molecular Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands, (2) Division of Molecular Carcinogenesis, The Netherlands Cancer Institute, Amsterdam, (3) Experimental Animal Pathology, The Netherlands Cancer Institute, Amsterdam

A variety of human cancer entities including breast and gastric carcinoma harbor genetic aberrations of the fibroblast growth factor receptor 2 gene (FGFR2), presumably resulting in oncogenic activity of FGFR2. In similarity of these observations, using a transposon-based insertional mutagenesis screen in mice, we previously identified Fgfr2 to be a candidate driver of invasive lobular breast carcinoma (ILC). Through mapping of transposon insertions, we now show that most insertions in Fgfr2 cluster to the intron upstream of the last exon (E16), which results in truncated Fgfr2 expression lacking E16. Consistently, ILC formation in mice is faithfully induced only through mammary-specific expression of truncated Fgfr2^{-E16}, while full-length Fgfr2 expression has little effect on tumorigenesis. The molecular consequences of FGFR2^{-E16} are strong potentiation of PI3K signaling and phosphorylation of S6 kinase, as compared to full-length FGFR2. Moreover, progressive deletion of the Fgfr2 3'-end and site-directed mutagenesis of E16 identifies a five-amino-acid motif highly critical for the suppression of both, S6 kinase phosphorylation and tumorigenesis in vivo. In human carcinomas, FGFR2 aberrations are frequently comprised of either fusion to a 3'-partner or amplification. Interestingly, fusions usually result in C-terminal FGFR2 truncation, while FGFR2 amplicons produce alternative transcripts lacking the last exon, both reminiscent of Fgfr2^{-E16} >oncogenicity. Indeed, in human tumor cell lines harboring FGFR2 fusions or amplifications, specific targeting of C-terminally truncated FGFR2 using RNAi strongly inhibits growth of these cells. Silencing full-length FGFR2, however, does not interfere with cell growth. Thus, we uncover a novel paradigm in oncogenic FGFR2 signaling that involves truncation of the C-terminal tail and propose therapeutic strategies that should target specific FGFR2 variants rather than blocking total FGFR2 signaling.

11

Metabolic vulnerabilities in lung tumor-initiating cells

Z. Wu (2), Z. Wang (1), W.L. Tam (1)

(1) Genome Institute of Singapore, Singapore, (2) Cancer Science Institute of Singapore, Singapore

Non-small cell lung cancer is one of the world's leading cause of cancer-related deaths. Elucidating metabolic vulnerabilities in lung tumor-initiating cells (TICs) would potentially allow development of novel targeted therapeutics that can reduce tumor growth and relapse in lung cancer patients. To understand specific metabolic alterations in lung TICs, an unbiased metabolomics investigation between lung TICs and its non-tumorigenic isogenic differentiated line was performed. We found significant differences in levels of several key metabolites under steady state conditions, and key protein expression changes that may account for metabolic differences observed in lung TICs. Here, we also performed genetic manipulation or pharmacological inhibition of these proteins to further reveal metabolic dependencies of lung TICs under specific in-vitro and in-vivo conditions.

12

CEP55 depletion can delay tumorigenesis through GSK3β/ β-Catenin/ Myc axis downstream of Akt signaling pathway

B. Rashidieh (2), A. Bain (1), A. Lopez (2), K. Khanna (1)

(1) Cancer and cell biology, QIMR Berghofer, Brisbane, Australia,
(2) Cancer and cell biology, QIMR Berghofer, Brisbane, Australia / School of Environment and Science, Griffith university, Australia

The heterogeneous nature of cancer is promoted by elevated levels of aneuploidy caused by genomic instability (GIN), which predisposes cells to malignant transformation. Cep55, a protein initially characterized by our laboratory as a cytokinesis regulator has also been shown to be an important regulator of GIN, and its overexpression correlates with aggressiveness, metastasis and poor prognosis in many tumor types. Moreover, our lab recently showed that loss of Cep55 sensitizes cancer cells to anti-mitotic agents, which could be a potential therapeutic strategy against Cep55-overexpressing tumors. Perturbation of CEP55 levels in in-vitro studies has also highlighted its roles in PI3K/Akt regulation, midbody fate, and stemness.

To better understand the role of Cep55 in the cancer context, we generated the first Cep55 Knockout (KO) mouse model. Interestingly, homozygous loss of Cep55 was late embryonically lethal, indicating a crucial physiological role in embryonic development. To understand the molecular causes underpinning this phenotype, we established mouse embryonic fibroblasts (MEFs) from Cep55 WT and KO embryos. This in-vitro model revealed increased cytokinesis failure leading to multinucleated cells and decreased proliferation in Cep55 KO MEFs, as a consequence of mitotic defects. To determine if Cep55 loss would perturb transformation ability, we transformed Cep55 WT and KO MEFs with E1A/Ras oncogenes. Strikingly, we found that anchorage-independent colony formation was reduced upon E1A/Ras Cep55 KO in colony formation assay and 3D culture. Similarly, loss of Cep55 abrogated tumor formation in NOD/SCID mice injected sub-cutaneously with E1A/Ras-transformed Cep55 KO MEFs, suggesting that CEP55 is essential for proper tumor formation. Immunoblotting of Cep55 WT and KO MEFs revealed roles of Cep55 in regulating GSK3β/ β-Catenin/Myc signaling, which may contribute to its role in regulating tumor formation. Altogether, our data suggest preliminary mechanistic evidence of Cep55 contribution to tumorigenesis through the GSK3β/ β-Catenin/ myc axis and Cep55 may be a useful molecular target to prevent the initiation and progression of cancer.

Key words: CEP55, Genomic instability, E1A/Ras, Akt, GSK3β/ β-Catenin/ Myc

13

Retinoblastoma with late metastatic spread, insights by means of DNA methylation classification of central nervous system tumours

S. Hofer (1), J. Diebold (2), P. Temming (3), D. Lohmann (4), J. Howell (5), J. Ecker (6), M. Beck Popovic (7)

(1) Medical Oncology, Luzerner Kantonsspital, Luzern, (2) Pathology, Luzerner Kantonsspital, Luzern, (3) Klinik für Pädiatrie III, Universitätsklinikum Essen, Essen, Germany, (4) Institut für Humangenetik, Universitätsklinikum Essen, Essen, (5) Augenklink, Luzerner Kantonsspital, Luzern, (6) Pädiatrische Hämatologie und Onkologie, Universitätsklinikum Heidelberg, Heidelberg, (7) Dept. Pediatrics and Pediatric Surgery, University Hospital Lausanne, Lausanne

Background: Retinoblastoma is a rare cancer of the infant retina, which forms when both RB1 alleles are functionally inactivated in a susceptible retinal cell, likely a cone photoreceptor precursor. Although pRB is expressed in virtually all cells, cone precursors have biochemical and molecular features that may sensitize to RB1 loss to enable tumourigenesis. Although more than 95% of patients survive 5 years, when treated adequately, late effects are a major concern and survivors bear a lifelong risk for developing second cancers.

Metastatic disease, many years after initial curative treatment, however, is a very rare manifestation and accordingly its diagnosis remains challenging. Primary disease and metastatic disease originate from molecularly highly related cells. The DNA methylation profile, depicting traits of the tumor's cell of origin, is largely conserved between primary and metastatic disease and therefore represents a feasible molecular marker for tumor classification and diagnosis. In recent years DNA methylation profiling has been successfully employed in research and clinical application for re-defining/refining classification of brain tumours.

Case report: A 20 - year old women presented to our clinic with moderate pain in her right calf and a growing lump in the right inguinal region. Her past medical history revealed unilateral heritable retinoblastoma with proven constitutional RB1 mutation (intron 18) diagnosed shortly after birth. The disease was treated with multi-agent chemotherapy and locally with photocoagulation. The following twenty years were uneventful.

Biopsy of a right inguinal lymph node revealed a small blue round cell tumour with Flexner-Wintersteiner rosettes and a high proliferation rate (Ki67 90%), highly characteristic of retinoblastom (Panel A). The DNA methylation profile of the tumour (determined by 850K MethylationEPIC BeadChip microarray, University of Heidelberg) perfectly matched the established methylation class "trilateral retinoblastoma". This reference methylation class includes cases of heritable ocular retinoblastoma. Furthermore, a copy number profile calculated from the DNA methylation data revealed several chromosomal aberrations typically observed in ocular retinoblastomas such as chromosome 1q gain and chromosome 16 loss (Panel B). Taken together, these molecular data strongly supported the diagnosis of a metastatic retinoblastoma.

PET-CT displayed activity in the right second toe (osteolysis in the basal phalanx), in lymph nodes along the right extremity and retroperitoneal up to the third lumbar vertebral body (Panel C). Four cycles of multi-agent chemotherapy (ARET 0321 protocol) according to the Children`s Oncology Group (COG) were followed by high dose chemotherapy and autologous stem cell rescue. PET positive lymph nodes were irradiated with 50 Gy and the affected toe was resected (basal phalanx with small blue round cells). More than two years later the patient remains well and in complete remission (PET-CT).

Conclusions: Heritable retinoblastoma bears a lifelong risk for developing second cancers, however, metastatic disease, is extremely rare. This manifestation has to be confirmed by means of molecular characteristics such as DNA methylation profiling, which has a substantial impact on diagnostic precision compared to standard methods.

14

Multi-faceted zinc finger RNA binding protein ZFP36L1 in monitoring and maintaining genome stability

N. Shaikh Solaiman, K. Surendranath, J. Murphy

Biomedical Sciences, University of Westminster, London

The emerging roles of RNA molecules in the maintenance of genome integrity and in DNA damage response poses several intriguing questions and is a significant area of study in the current research landscape. RNA-binding proteins (RBPs) bind to RNA molecules through RNA-binding domains and play a significant role in the regulation of gene expression at the post-transcriptional level including splicing, polyadenylation, and mRNA decay. Furthermore, the ZFP36 family of RBPs promote mRNA degradation through the binding of their conserved zinc finger motifs to Adenine Uridine (AU) rich elements in the 3' untranslated regions of mRNA molecules. Recently, RBPs have been increasingly shown to be directly involved in the DNA damage response and in the maintenance of genome integrity by interacting with nascent RNAs and DNA repair factories. Unlike the established roles of ZFP36 family of RBPs in mRNA regulation, the precise link of these proteins to genomic instability is still largely unknown. A variant of the ZFP36 family, the ZFP36L1 protein is differentially expressed in various cancer cell types as observed by others and our laboratory. A recent large-scale study on breast cancer patients identified ZFP36L1 as one of the top ten breast cancer driver genes including BRCA1 and BRCA2. Strikingly, our studies indicate a strong correlation between ZFP36L1 and replication stress. We will be presenting data showing the nuclear, nucleolar, morphological and signalling changes that occur during RNAi-mediated transient knock down and CRISPR knockout of ZFP36L1.The functional implications of these findings and future perspectives will also be discussed.

15

CtIP synergizes with BRCA1 to protect reversed forks from nucleolytic degradation

S. Przetocka, A. Sartori

Institute of Molecular Cancer Research, University of Zurich, Switzerland

Various cellular mechanisms cooperate to ensure faithful DNA replication and maintain genome stability. However, replication forks are frequently challenged by exogenous or endogenous DNA damaging agents. Homologous Recombination (HR) and the Fanconi Anemia (FA) pathways act in collaboration in order to secure fork stabilization and promote fork recovery. Human CtIP, in conjunction with the MRE11 nuclease complex, is most widely recognized for its essential function in promoting DNA-end resection to initiate HR. Our recent work now establishes that CtIP protects replication forks from nucleolytic degradation in an MRE11-independent manner. Specifically, upon treatment with hydroxyurea (HU), a drug that transiently arrests replication forks by depleting the nucleotide pool, we observe that CtIP deficiency triggers DNA2-mediated over-resection of nascent DNA strands. Moreover, our data indicates that CtIP preserves the integrity of stalled forks that have undergone fork reversal, a process promoted by the SNF2-family of DNA translocases and by RAD51. Unexpectedly, we find that CtIP takes advantage of its proposed nuclease activity to keep DNA2 in check and stabilize stalled replication forks. In an attempt to decipher the molecular network collaborating with CtIP in fork protection, we uncover a non-epistatic relationship between CtIP and BRCA1, which is critical to maintain genome stability. Collectively, our results highlight a previously unrecognized and important role of CtIP in response to DNA replication stress that, if lost, may facilitate tumorigenesis. We are currently expanding on these findings and evaluating the functional interplay between CtIP and DNA helicases/nucleases in promoting fork stabilization.

16

Dosimetric impact of multileaf collimator leaf width according to sophisticated grade of technique in the IMRT and VMAT planning for pituitary adenoma lesion

S. M. Chae (1), K. W. Lee (1), S. H. Son (2)

(1) Radiation Oncology, Cheju Halla General Hospital, Jeju, Korea, (2) Radiation Oncology, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

Purpose: We analyzed the difference in the dosimetric effect between 5-mm and 2.5-mm multileaf collimator (MLC) leaf width according to the sophisticated grades of intensitymodulated radiotherapy (IMRT) and volumetric-modulated arc therapy (VMAT).

Methods: Nineteen patients with pituitary adenomas were selected for this study. The treatment plans were performed according to the size of the MLC (5-mm and 2.5-mm MLC), the type of technique (IMRT and VMAT), and the sophisticated grades of each technique (5-field, 9-field, 13-field, 17-field technique in IMRT and 1-arc and 2-arc techniques in VMAT). The downsizing effects of MLC leaf width were analyzed using target volume coverage (TVC), conformity index (CI), dose gradient index (GI), and normal tissue difference 70% isodose line and 50% isodose line.

Results: Upon replacing the 5-mm MLC with the 2.5-mm MLC, TVC and CI improved by 1.30% and 1.36%, respectively, in total plans. The TVC and CI improved by 1.68% and 1.67% in IMRT, respectively, and by 0.54% and 0.72% in VMAT, respectively. TVC improved by 2.53%, 1.82%, 1.34%, and 0.94%, and CI also improved by 2.70%, 1.81%, 1.24%, and 0.94%, in 5-field, 9-field, 13-field, and 17-field IMRT, respectively. TVC improved by 0.66% and 0.43%, and CI also improved by 0.93%, and 0.52% in 1-arc and 2-arc VMAT, respectively.

Conclusion : Regarding the target coverage, there were dosimetric benefits of a smaller MLC leaf width. However, the downsizing effect of the MLC leaf width decreased with the use of a more precise RT technique and a more sophisticated grade of the same technique.

Keywords: multi-leaf collimator, radiosurgery, intensity-modulated radiotherapy, volumetric modulated arc therapy

17

Hunting for Tumor Initiating Cells by Visualizing mir145 in Ewing Sarcoma

T. Keskin, S. Cornaz, P. Marten, C. Fusco, N. Riggi, I. Stamenkovic

Institute of Pathology, CHUV, Lausanne

Tumors are heterogeneous entities, composed of different cellular subpopulations bearing different genetic and epigenetic backgrounds. Within this diversity, Cancer Stem Cells (CSCs) are suggested to represent the tumor driving force, displaying tumor initiation, as well as responsibility for tumor recurrence, chemotherapy resistance and metastasis. Therefore, the development of CSC-targeted therapies requires detailed identification, isolation and analysis of this cellular subpopulation from primary human tumors.

A wide panel of cell surface markers has been used to detect and isolate CSCs in different tumors. Although some of them have shown to be successful, none of the known markers was able to uniquely mark CSCs. In addition, the expression of currently used markers may have little or no direct functional role in carcinogenesis. All of these shortcomings indicate the necessity to identify CSCs using a different approach, which could reliably reflect their key intrinsic properties.

According to our previous studies, mir145 is downregulated in Ewing Sarcoma (EwS) tumor initiating cells. Moreover, its overexpression correlates with CSC differentiation and loss of tumorigenicity. Thus, a reporter system able to detect intracellular mir145 expression levels may provide an effective and robust strategy to distinguish cells with different degrees of plasticity in a tumor bulk.

In the present work, we generated an inducible green fluorescent protein (GFP)-based miRNA reporter system, which enables the identification and purification of primary EwS cells displaying different degrees of mir145 expression and activity. Our results showed that this reporter assay is able to distinguish and isolate EwS primary tumor cells with different tumorigenic capacities based on their endogenous mir145 expression levels. The results from this project may contribute to a more detailed understanding of tumor initiating cells in Ewing Sarcoma, which in turn could lead to the development of new therapeutic strategies targeting EwS CSCs.

18

Tumor initiation capacity and therapy resistance are differential features of EMT-related subpopulations in the NSCLC cell line A549

T. M. Marti (1), C. C. Tièche (1), E. D. Bührer (2), Y. Gao (1), N. Hobi (3), S .A. Berezowska (4), K. Wyler (5), L. Froment (1), S. Weis (6), R. W. Peng (1), R. Bruggmann (5), P. Schär (6), M. A. Amrein (2), S. R. Hall (1), P. Dorn (1), G. Kocher (1), C. Riether (2), A. Ochsenbein (2), R.A. Schmid (1)

(1) Division of General Thoracic Surgery, Inselspital, Bern University Hospital, Bern, Switzerland, (2) Department of Medical Oncology, Inselspital, Bern University Hospital, Department of Clinical Research, University of Bern, Bern, Switzerland, (3) Institute of General Physiology, University of Ulm, Ulm, Germany, ARTORG Center for Biomedical Engineering Research, Organs-on-Chip Technologies, University of Bern, Bern, Switzerland, (4) Institute of Pathology, University of Bern, Bern, Switzerland, (5) Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland, (6) Department of Biomedicine, University of Basel, Basel, Switzerland

Background: More than 80% of lung tumors are non-small-cell lung cancers (NSCLC). Cancer stem cells (CSCs) are characterized by an increased expression of stemness-associated genes, and drive tumor initiation and therapy resistance.
Cell lines are essential tools to standardize and compare experimental findings in basic and translational cancer research. Here, we identified and comprehensively characterized three morphologically distinct cellular subtypes in the NSCLC cell line A549.

Methods: Whole genome mRNA expression was analyzed by Illumina HiSeq3000 2x 150 bp paired end sequencing. Protein expression was analyzed by multicolor flow cytometry and immunofluorescence microscopy. Mouse studies were conducted in accordance with Institutional Animal Care and Ethical Committee-approved animal guidelines and protocols. Immunohistochemical staining was performed using an automated Bond III immunostainer.

Results: Subtype-specific cellular morphology is maintained during short-term culturing, resulting in the formation of morphologically distinct colonies (holoclonal, meroclonal and paraclonal colonies). A549 holoclone cells were characterized by an epithelial and stem-like phenotype, paraclone cells featured a mesenchymal phenotype whereas meroclone cells were phenotypically intermediate. Cell-surface marker expression of subpopulations changed over time, indicating an active epithelial-to-mesenchymal transition (EMT), in vitro and in vivo. EMT has been associated with the overexpression of the immunomodulators PD-L1 and PD-L2, which were overexpressed in para- versus holoclone cells, respectively. We found that DNA methylation is involved in epigenetic regulation of marker expression. Holoclone cells were extremely sensitive to cisplatin- and radiotherapy in vitro, whereas paraclone cells were highly resistant. Xenograft tumor formation capacity was highest in holoclone cells.

Conclusions: Our protocol for isolating subpopulations from the A549 line might provide a unique system to study the network of stemness, tumor initiation capacity, invasive and metastatic potential and therapy resistance.

19

IL-33/ST2 signaling promotes the survival and proliferation of AML1/ETO leukemic stem cells

P. Näf (1), R. Radpour (1), C. Riether (1), A. F. Ochsenbein (2)

(1) Department of BioMedical Research, Tumor-Immunology, Bern, (2) Department of Medical Oncology, Inselspital, Bern

Interleukin (IL)-33 is an alarmin released upon cell necrosis and binds to the heterodimeric receptor ST2/IL1RaP, which is expressed on a subset of immune and epithelial cells. BCR/ABL transformation in chronic myeloid leukemia (CML) stem cells was shown to induce ST2 expression and propel malignant cell growth. However, the role of IL-33/ST2 signaling in acute myeloid leukemia (AML) is currently unknown.
Gene expression analysis of human bulk and CD34+CD38+ AML samples harboring variety of translocations revealed that ST2 was particularly upregulated on AML1/ETO transformed AML cells, but not expressed on other AML subtypes and healthy hematopoietic stem cells (HSCs). We next generated an in vivo AML model by transfecting murine hematopoietic stem cells with an AML1/ETO lentiviral construct. AML/ETO transformed leukemic stem cells (LSCs) highly expressed ST2 and showed increased colony formation in recombinant IL-33 (rIL-33) conditioned methylcellulose-based culture assays when compared to controls.
Interestingly, amongst all screened human AML1/ETO LSCs a heterogeneity in ST2 expression was observed. Molecular profiling of the LSCs revealed a negative correlation of ST2 gene expression and key

genes of the Notch pathway. Blocking the Notch pathway in the ST2lowAML1/ETO LSCs using siRNA revealed that ST2 protein expression could be restored. Furthermore, siRNA induced gain of ST2 expression also restored in vitro colony formation capacity in the presence of rIL-33.
Taken together, these data provide evidence that IL-33/ST2 signaling plays a disease promoting role in AML1/ETO transformed AML LSCs. Further investigations will focus on detailed signaling mechanisms and propose new therapeutic strategies to treat AML.

20

Gain fat – Lose metastasis: Converting invasive breast cancer cells into adipocytes inhibits cancer metastasis

D. Ishay Ronen (1), M. Diepenbruck (1), R.K.R. Kalathur (1), N. Sugiyama (1), S. Tiede (1), R. Ivanek (1), G. Bantug (2), M. F. Morini (1), C. Hess (2), G. Christofori (1)

(1) Department of Biomedicine, University of Basel, Switzerland, (2) University Hospital Basel, Department of Biomedicine, University of Basel, Switzerland

Introduction: Cancer cell plasticity facilitates the development of therapy resistance and malignant progression. Cancer cell plasticity is of a dynamic nature and can be the result of changing cues in the microenvironment. An epithelial-mesenchymal transition (EMT) seems to play a major role in facilitating cell plasticity in cancer and allows cancer cells to escape chemotherapies and targeted therapies by dedifferentiation and signaling adaption processes. In this study, we hypothesize that cancer cell plasticity is necessary for cancer dissemination but can be directly targeted and inhibited by forcing the trans-differentiation of EMT-derived breast cancer cells into post-mitotic adipocytes.

Material and method: Using established EMT models of murine mammary cancer cells, we studied the adipogenesis trans-differentiation potential of EMT-derived cancer cells versus their epithelial ancestors. We utilized established methods from adipogenesis studies to morphologically and functionally characterize cancer-derived adipocytes in vitro. The kinetics and gene expression regulation during cancer cell adipogenesis were analyzed based on RNA sequencing. Analysis of TGFβ signaling pathway activation during adipogenesis of cancer cells revealed clinically relevant targets for the induction of adipogenesis in vivo.

Delineation of the molecular pathways underlying such trans-differentiation has motivated a combination therapy with a MEK inhibitor and a PPARγ ligand in various mouse models of murine and human breast cancer in vivo. Direct cancer cell- adipogenesis, primary tumour invasiveness and metastasis formation were analyzed. To test the effect of adipogenic trans-differentiation as therapeutic option for breast cancer, we used apatient-derived xenograft (PDX) mouse model of human tripple-negative breast cancer in preclinical settings.

Results and discussion: Here, we demonstrate that cancer cell plasticity can be exploited therapeutically by a trans-differentiation approach. We target cancer cell plasticity by forcing the irreversible conversion of EMT-derived breast cancer cells into post-mitotic and functional adipocytes. Epithelial cancer cells lack this potential, supporting the notion that an EMT coincides with increased cell plasticity. In various preclinical mouse models, a combination therapy provoked the conversion of invasive and disseminating cancer cells into post-mitotic adipocytes leading to the repression of primary tumor invasion and metastasis formation.

Conclusion: The results underscore the pivotal role of cancer cell plasticity in malignant tumor progression and reveal the therapeutic potential that lies in the inhibition of cellular plasticity, for example by forcing post-mitotic adipogenesis.

21

Rates of hepatocellular carcinoma within two years after start of treatment for chronic hepatitis C

F. Karbeyaz (1), A. R. Siebenhüner (2), B. Müllhaupt (1), B. Misselwitz (1), T. Winder (2), J. C. P. Mertens (1)

(1) Gastroenterology and Hepatology, University Hospital Zurich, Switzerland, (2) Oncology Department, Universitätsspital Zurich

Background: Direct antiviral agents (DAA) have revolutionized the treatment of chronic hepatitis C (CHC) and are now standard of care for CHC patients. DAA have replaced previous therapy regimes based on PEG-interferon (PEG-IFN) and Ribavirin (RBV) which were poorly tolerated and much less effective. An increased risk for hepatocellular carcinoma in patients with liver cirrhosis on the basis of untreated CHC is known (3-5% per year). To date the correlation between eradication of viral hepatitis respectively the viral load and the risk of HCC development in CHC patients is not well understood but several studies describe an increase in HCC incidence after DAA treatment. This retrospective study aims to analyze effects of DAA or PEG-INF/ RBV treatment on the development of HCC.

Methods: The Swiss hepatitis C cohort study (SCCS) prospectively collects clinical information from patients infected with HCV. The study was approved by all ethics commitees and participants provided written consent. We performed a retrospective analysis of 233 consecutive patients that received treatment with PEG-IFN/RBV and 259 patients that received DAA treatment for CHC in our institution. HCC viral load, genotype, treatment type, duration and outcome, fibrosis grade, age, gender, BMI and serum albumin were retrieved. A Cox-proportional hazard model was fit to compare survival in patients treated with PEG-IFN/RBV and patients that received DAA regarding HCC, need for liver transplantation and death. Diagnosis of HCC followed established guidelines by the European Association for the Study of the Liver (EASL). Patients were considered negative for HCC if dedicated imaging (either abdominal ultrasound or contrast-enhanced MRI) was without evidence for HCC. For all other patients HCC state was considered unknown. Patients within the HCV cohort received annual ultrasonography of the liver while patients with known cirrhosis received biannual surveillance by ultrasound. Suspicious lesions were generally followed by MRI. Cirrhosis was defined either by biopsy or fibroscan or according to clinical and ultrasound criteria (i.e. severe liver disease with clear radiographic or endoscopic signs of portal hypertension).

Results: 8 HCC cases among patients treated with DAA were observed compared to 17 cases after PEG-INF/RBV treatment. A univariate Cox-proportional hazard model revealed a significant increase in the development of HCC upon DAA treatment compared to PEG-INF/ RBV (hazard ratio, HR 5.5, p=0.047) within the first two years after treatment. In a multivariate Cox-proportional hazard model, age (HR 1.07/ year, p=0.004), SVR (HR 0.23, p=0.002), cirrhosis (HR 2.75, p=0.024) and DAA treatment (HR 5.6, p=0.04) were significant predictors for HCC.
After DAA treatment, time to HCC diagnosis was significantly shorter than after PEG-INF/ RBV (0.84 years, range: 0.5-2.1 vs. 6.6 years, range: 2.3-15). Occurrence of HCC was highest within the first year after DAA treatment (5 cases) but lower in the second year (2 cases) and third year (1 case) after first DAA exposure. In contrast, death and need for liver transplantation did not differ between the PEG-IFN/RBV and DAA group.

Conclusion: Our data show high rates of HCC early after DAA but not after PEG-IFN/ RBV treatment. In contrast to earlier studies using epidemiological comparisons, our study limited confounders by directly comparing incidence of HCC after PEG-INF/RBV and DAA treatment within the same institution. Close surveillance for HCC occurrence seems warranted especially within the first year after start of DAA treatment.

22

Reinterpreting EMT as a state of dynamic cellular plasticity contemporaneous to distinct cancer subtypes

D. Ishay Ronen* (1), C. K. Y. Ng* (2), M. Saxena (1), A. Zviran (3), N. Novershtern (3), J. Hanna (3), G. Christofori (1)

(1) Department of Biomedicine, University of Basel, Switzerland, (2) Institute of Pathology, University Hospital of Basel, Basel, Switzerland, (3) Department of Molecular Genetics, Weizmann Institute of Science, Israel

Epithelial-to-mesenchymal transition (EMT) traditionally implies a shift in cell identity. Yet, EMT-like changes are observed in a variety of cell types that do not correspond to a linear conversion (e.g. embryogenesis, cell reprogramming into induced pluripotent stem cells (iPSC) and EMT in triple negative breast cancer). Here we used RNA sequencing data from several cellular models undergoing EMT-like changes to uncover shared cellular mechanisms with the aim to define a plasticity signature. The analysis revealed two plasticity states, a 'full-EMT' state and a 'partial-EMT' state, with distinct gene expression profiles that correspond to two different aggressive breast cancer subtypes. ~25% of the genes are shared between the two states but show inverse expression patterns, highlighting the dynamic nature of cellular plasticity. The results emphasize the mutual traits of EMT and cellular plasticity in cancer, regardless of cell identity and original cellular characteristics.

23

Identification of potential therapeutic targets in prostate cancer driven by different genetic backgrounds by polysome profiling

D. Brina (1), N. Parhizgari (1), M. Mirenda (2), A. Calcinotto (1), N. Delaleu (1), A. Alimonti (1)

(1) IOR Institute of Oncology Research, Bellinzona (2) Molecular Oncology, IOR Bellinzona

Prostate cancer is the second most commonly occurring cancer in men and the fourth most commonly occurring cancer overall. In 2018 the new cases are 1.3 million. There is an urgent need for development of new effective drug strategies that can counteract the onset of resistance to androgen deprivation therapy and metastasis.
Tumor microenvironment plays a fundamental role in the progression of cancer as demonstrated by the emergence of cancer immunotherapy. Recently, it has been demonstrated that anti-PD1 immunotherapy is effective in the treatment of prostate cancer carrying DNA repair mutations. Thus, the genetic background can shape the immune response leading to an immunosuppressive microenvironment and cancer immunotherapy can be tailored for specific genetic alterations.
Our aim is to identify novel secreted or transmembrane proteins expressed on the surface of the tumor cells from different genetic backgrounds that mediate the recruitment or skewing of myeloid cells, eliciting immunosuppression and tumor escape from the immune surveillance.
In order to characterize the immune landscape of preclinical models of prostate cancer driven by different genetic alterations, we performed polysome profiling of normal prostate and prostate tumor of Pten$^{pc-/-}$, Pten $^{pc-/-}$; TMPRSS2-ERG, Pten $^{pc-/-}$; CDCP1+, Pten $^{pc-/-}$; TIMP1 $^{-/-}$ and Pten $^{pc-/-}$; Trp53 $^{pc-/-}$. Pten and Trp53 loss and TMPRSS2-ERG fusion are among the most common genetic alterations in prostate cancer. Timp1 loss and Cdcp1 overexpression in prostate cancer has been identified in the lab. These mouse models are characterized by different aggressiveness and tumor infiltration: Pten$^{pc-/-}$ and Pten $^{pc-/-}$; TMPRSS2-ERG mice are late and early onset prostate cancer, respectively; Pten $^{pc-/-}$; CDCP1 $^{pc+}$ and Pten $^{pc-/-}$; TIMP1 $^{-/-}$ are metastatic in the late phase of the disease. The Pten $^{pc-/-}$; Trp53 $^{pc-/-}$ model is lethal, with a median survival of 6 months.
We matched the gene expression profiling from prostate cancer to the signature obtained from bone marrow-derived monocytic Ly6C^{high}; Ly6G^{neg} and granulocytic Ly6C^{int}; Ly6G^{high} myeloid derived suppressor cells (MDSCs), in order to identify proteins upregulated in prostate cancer that can modulate MDSCs through the binding to their specific

receptor. In order to investigate in vivo the function of the identified receptors and to establish whether they might be therapeutic targets for cancer immunotherapy, a HoxB-mediated immortalized Cas9 bone marrow-derived cell line has been established and $Pten^{pc-/-}$ mice had been reconstituted with the bone marrow knockout for the specific receptor.
We are currently investigating a set of receptors that we found up-regulated in MDSc and the potential protective benefit of their inhibition in preclinicals models of prostate cancer.
The identification of novel receptors involved in the recruitment or skewing of immunosuppressive myeloid cells into the tumor will allow possibly to design new immunotherapy strategies that, combined with exisisting chemotheraphy, might ameliorate the efficacy of prostate cancer treatment.

24

Identification of fatal outcome in a childhood nasopharyngeal carcinoma patient by protein expression profiling

M. E. Saeed (1), R. Mertens (2), R. Handgretinger (3), T. Efferth (1)

(1) Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, (2) Section for Pediatric Hematology, Oncology and Stem Cell Transplantation, Department of Pediatric and Adolescent Medicine, (3) Department of Paediatric Haematology/Oncology, Children's University Hospital

Nasopharyngeal carcinoma (NPC) is a rare disease in children with good prognosis and high cure rate. Nevertheless, certain patients have an unfavorable prognosis due to development of refractory NPC that is unresponsive to any therapeutic strategies. The current study reports a case of a 17 years-old female with non-keratinizing NPC type IIb (T2N0M0), who passed away as a consequence of resistance to chemo-, radio- and β-interferon therapy, and to an allogenic stem cell transplantation. In order to identify factors that lead to treatment failure and fatal outcome, immunohistochemical analyses of different tumor biomarkers and hierarchical cluster analysis were performed and compared with those of eight other patients with NPC who experienced complete remission following conventional therapy. Hierarchical cluster analysis of the immunohistochemical results clearly demonstrated that staining for immunological factors (CD4, CD8 and CD56) distinguished this patient from the others. To further investigate a potential role of the immune system, lymphocytic infiltration was assessed in tumor tissue by evaluation of hematoxylin and eosin-stained tumor sections. Indeed, no tumor infiltrating lymphocytes (TILs) were observed in this NPC case, while 7 out of 8 of the other NPC samples contained variable TIL amounts. The view that immunodeficiency of the patient may be a factor in the fatal outcome of treatment is supported by the fact that this patient with NPC was not positive for Epstein-Barr virus markers and also infected by several other viruses and fungi (herpes simplex virus, human herpes virus 6, Varicella zoster virus, and Candida). In conclusion, the investigation of rare NPC cases with poor prognosis may provide an improved understanding of the molecular mechanisms involved in refractory tumors and identification of novel potential therapeutic targets for NPC in the future.

25

Genome-wide silencing screen in mesothelioma cells reveals that loss of function of BAP1 induces chemoresistance to ribonucleotide reductase inhibition: implication for therapy

A. Okonska (1), S. Bühler (1), V. Rao (1), M. Ronner (1), M. Blijlevens (2), I. H. Van der Meulen-Muileman (2), R. X. De Menezes (3), E. F. Smit (4), W. Weder (5), R. A. Stahel (6), L. Penengo (7), V. W. Van Beusechem (2), E. Felley-Bosco (1)

(1) Laboratory of Molecular Oncology, Division of Thoracic Surgery, USZ, 8091 Zurich, Switzerland, (2) Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands, (3) Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, (4) NKI, Amsterdam, The Netherlands, (5) Division of Thoracic Surgery, University Hospital Zurich, 8091 Zurich, Switzerland, (6) Cancer Center Zurich, University Hospital Zurich, 8091 Zurich, Switzerland, (7) Institute of Molecular Cancer Research, University of Zurich, 8057 Zurich, Switzerland

Objectives: BRCA1 associated protein 1 (BAP1) is mutated or deleted in about 50% of the malignant pleural mesothelioma (MPM) cases. Recent studies on BAP1 function demonstrate its broad role in many cellular processes such as chromatin modification via H2A deubiquitination, transcriptional regulation, and DNA repair. Understanding the molecular function of BAP1 in MPM contributes to development of targeted therapy depending on BAP1 status in MPM.

Methods: A genetically engineered model was established expressing either functional or non-functional BAP1 in the same genetic background. Isogenic cell lines were then characterised via H2A deubiquitination and response to olaparib. Whole-genome siRNA screens were performed assessing impaired survival comparing the BAP1-proficient vs. BAP1-deficient MPM cells. Hits were validated by survival assay after silencing with distinct siRNA sequences and verification of knockdown via Western blotting. Cytotoxity induced by gemcitabine and hydroxyurea were performed in 3D (spheroids) on a panel of BAP1 WT and BAP1 mut/del cell lines. DNA damage response was assessed via western blotting by detecting yH2AX, pKAP1 and p-p53 levels. Upregulation of RRM2 upon induced replication stress was tested in NCI-H2452 cell line reconstituted with either EV, or BAP1 WT, or BAP1 C91A (catalytically dead mutant) and then via siRNA-induced knock-down of BAP1 in SPC11 cell line (BAP1 WT). The rescue experiments were performed in BAP1 WT SPC111 cell line with siRNA-induced knockdown of BAP1 in SPC11 cell line (BAP1 WT).

Results: The whole-genome siRNA screen revealed 11 hits with $p<0.05$ and FDR<0.05. Unexpectedly, two actionable targets, namely RRM1 and RRM2, were revealed and validated in BAP1-proficient cells. RRM1 and RRM2 inhibition mediated by gemcitabine or hydroxyurea respectively was more profound in BAP1-proficient MPM cell lines. Both cell line groups demonstrate an upregulation of RRM2 upon gemcitabine or hydroxyurea treatment, but this effect is much more dramatic in BAP1 mut/del cell lines. In concordance, we observed an increased lethality in NCI-H2452 cell line reconstituted with BAP1 WT but not with a catalytic dead C91A mutant upon gemcitabine or hydroxyurea treatment. Moreover, we observe more modest up-regulation of RRM2 in NCI-H2452-BAP1 WT spheroids than in spheroids expressing either EV or C91A mutant. Finally, BAP1 knockdown in BAP1-proficient cell line reverts this effect, where in replication stress conditions RRM2 levels are elevated upon BAP1 silencing.

Conclusion: We found a BAP1 status dependency in MPM, which seems to be rooted in a direct or indirect regulation of RRM2 and/or RRM1 expression on transcriptional level by BAP1 during replication stress. Overall, these observations give new insights into BAP1 function in replication stress and reveal a novel aspect of therapeutic potential in MPM depending on BAP1 status, thus MPM patients could be stratified depending on BAP status of a tumor and treated with gemcitabine or hydroxyurea.

26

Oridonin Targets Multiple Drug-Resistant Tumor Cells as Determined by in Silico and in Vitro Analyses

O. Kadioglu (1), M. Saeed (1), V. Kuete (1), H.J. Greten (2), T. Efferth (1)

(1) Johannes Gutenberg University, Institute of Pharmacy and Biochemistry, Pharmaceutical Biology Department, Mainz, Germany, (2) Heidelberg School of Chinese Medicine, Heidelberg, Germany

Overcoming drug resistance is an invaluable approach to identify novel anticancer drugs that have the potential to bypass or overcome resistance to established drugs and to substantially increase life span of cancer patients for effective chemotherapy, since drug resistance is one of the main reasons of chemotherapy failure. In the present study, we evaluated the cytotoxicity of oridonin, a diterpenoid isolated from Rabdosia rubescens, towards a panel of drug-resistant cancer cells overexpressing ABCB1, ABCG2, expressing mutant EGFR with a deletion at the ligand binding domain or with TP53 deletion. Interestingly, oridonin revealed lower degree of resistance than the control drug, doxorubicin. Oridonin can interact with Akt/EGFR pathway proteins with comparable binding energies and similar docking poses as the known inhibitors. Stable conformation of oridonin docking pose on Akt kinase domain was validated by molecular dynamics simulation. Western blot experiments clearly revealed dose-dependent downregulation of Akt and STAT3. Pharmacogenomics analyses pointed out genes that can confer sensitivity and resistance to oridonin. In conclusion, oridonin bypasses major drug resistance mechanisms, targets Akt pathway and might be effective towards drug refractory tumors. The identification of oridonin-specific gene expressions may be useful for the development of personalized treatment approaches.

27

Repurposing of bromocriptine for cancer therapy

E. J. Seo (1), Y. Sugimoto (2), H. J. Greten (3), T. Efferth (1)

(1) Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Staudinger Weg 5, 55128 Mainz, Germany, (2) Division of Chemotherapy, Faculty of Pharmacy, Keio University, Tokyo, Japan, (3) Abel Salazar Institute of Biomedical Sciences, University of Porto, Porto, Portugal

Bromocriptine is an ergot alkaloid and dopamine D_2 receptor agonist used to treat Parkinson´s disease, acromegaly, hyperprolactinemia and galactorrhoea, and more recently diabetes mellitus. The drug is also active against pituitary hormone-dependent tumors (prolactinomas and growth-hormone producing adenomas). We investigated, whether bromocriptine also inhibits hormone-independent and multidrug-resistant (MDR) tumors.

We found that bromocriptine was cytotoxic towards drug-sensitive CCRF-CEM, multidrug-resistant CEM/ADR5000 leukemic cells as well as wild-type or multidrug-resistant ABCB5-transfected HEK293 cell lines, but not sensitive or BCRP-transfected multidrug-resistant MDA-MB-231 breast cancer cells. Bromocriptine strongly bound to NF-κB pathway proteins as shown by molecular docking and interacted more strongly with DNA-bound NF-κB than free NF-κB, indicating that bromocriptine may inhibit NF-κB binding to DNA. Furthermore, bromocriptine decreased NF-κB activity by a SEAP-driven NF-κB reporter cell assay. The expression of MDR-conferring ABC-transporters (ABCB1, ABCB5, ABCC1, ABCG2) and other resistance-mediating factors (EGFR, mutated TP53, IκB) did not correlate with cellular response to bromocriptine in a panel of 60 NCI cell lines. There was no correlation between cellular response to bromocriptine and anticancer drugs usually involved in MDR (e.g. anthracyclines, Vinca alkaloids, taxanes, epipodophyllotoxins and others). COMPARE analysis of microarray-based mRNA expression in these cell lines revealed that genes from various functional groups such as ribosomal proteins, transcription, translation, DNA repair, DNA damage, protein folding, mitochondrial respiratory chain and chemokines correlated with cellular response to bromocriptine.

Our results indicate that bromocriptine inhibited drug-resistant tumor cells with different resistance mechanisms in a hormone-independent manner. As refractory and otherwise drug-resistant tumors represent a major challenge to successful cancer chemotherapy, bromocriptine may be considered for repurposing in cancer therapy.

28

Loss of cIAP1 in the tumor microenvironment disfavors cancer metastasis

L. A. Z. Vasilikos (1), K. A. Y. Hänggi (2), L. M. Spilgies (1), W. W. L. Wong (1)

(1) Experimental Immunology, University of Zurich, Zurich
(2) Immunology, H. Lee Moffitt Cancer Center, Tampa

Cancer patients usually succumb to their disease due to metastasis and not the primary tumor. Data from embryonic studies of ciap1/2 deletion and the use of Smac mimetics suggest that endothelial cells may require cIAPs for survival and permeability regulation. However, little is known about the role of cIAP1/2 in the tumor microenvironment during metastasis. Using birinapant, an inhibitor of cIAP1/2, and primary endothelial cells, we show that the loss of cIAP1 reduces permeability as well as tumor cell transmigration. cIap1$^{-/-}$ endothelial cells were unresponsive to extracellular cues to alter cytoskeleton signaling permitting permeability or extravasation. To mimic the latter stages of metastasis, namely extravasation and colonization, mouse tumor cells were tail vein injected. Surprisingly, the loss of cIAP1 but not cIAP2 reduced the number of tumor nodules formed in the lung. On the contrary, no differences in the tumor growth were seen in ciap1 or ciap2 deficient mice when the cells were injected subcutaneously suggesting tumor rejection is not the reason for reduced tumor nodules in the lung. In agreement, loss of cIAP1 in the hematopoietic compartment did not affect circulating tumor cell viability, extravasation or tumor nodule growth. Specific loss of cIAP1 using tamoxifen driven VE-cadherin cre identified the vascular endothelium as the cause for the reduction of nodules in the lung. Interestingly, the co-loss of TNFR1 but not TNFR2 with cIAP1 resulted in a similar tumor nodule load compared to wild type. Despite the involvement of the TNFRs, evidence implicating cell death was absent. Remarkably, administration of birinapant prior to tumor challenge caused the reduction of tumor metastasis by altering the endothelial barrier and inhibiting the ability of tumor cells to extravasate.

29

A CRISPR/Cas9-mediated Screening for F-box domain-containing E3 Ligase family in Intestinal Stem Cell Organoids

S. M. H. Kashfi, N. Jinks, A. Shams Nateri

Cancer Genetics & Stem Cell Group, Cancer Biology Unit, Division of Cancer and Stem Cells, School of Medicine, University of Nottingham, UK

Purpose: Organoid culture faithfully reproduces in vitro the in vivo structure of the intestinal/colon epithelium and allows elucidating molecular mechanisms underlying the regulation of stem cell compartment that, if altered, may lead tumourigenesis.
Now, in the present study, we applied the CRISPR/Cas9-mediated engineering method for screening of 34 F-box domain-containing E3-ligases in intestinal organoids. As the biological activity of the majority of F-box proteins (FBPs) in colorectal cancer (CRC) is elusive, exploring the function of FBPs family would further provide promising information on their role in CRC tumourigenesis.

Methods: The gRNA library of F-box proteins was validated using sequencing, restriction enzyme digestion and generation of stable cell lines in cultured cells. The lentivirus containing CRISPR gRNA were generated for further transduction of intestinal organoids. Furthermore, the transgenic organoids line expressing cas9 were generated through lentiviral transduction.

Results: Following transduction of cas9 organoids, the phenotypical/morphological alterations of the K/O organoids were evaluated for selecting the candidates and further downstream analysis.
Among 34 K/O organoids for F-box proteins, the K/O FBXL18 and K/O FBXL17 showed significant morphological changes in comparison to their controls. The K/O FBXL18 organoids failed to differentiate and the following organogenesis as expanded mature control counterparts. The suppression of proliferation and increase in apoptosis were also detected in K/O FBXL17 organoids.

Downstream analysis of the K/O FBXL18 in wound healing scratch assay showed a significant decreased movement of cells migration in comparison to control Cas9 DLD-1group ($P < 0.01$, respectively). Additionally, the colony formation assay also showed the significant decrease in the number of colonies compared with the Cas9 DLD-1 control group ($P < 0.01$).

Conclusion: FBPs K/O intestinal organoids led to phenotypical alterations that could be associated with proliferation and differentiation. CRISPR/Cas9 organoid mutagenesis can be utilized for:
- Screening of candidate genes for possible involvement in tumorigenesis.
- To validate findings from other model systems in an ex vivo setting.

30

The chromatin remodeler CHD4 regulates super-enhancer driven gene expression together with BRD4 and is essential for fusion positive pediatric sarcomas

J. Graca Marques (1), B. Gryder (2), B. Pavlovic (1), Y. Chung (1), Q. Ngo (1), Y. Song (2), K. Holste (1), D. Laubscher (1), M. Wachtel (1), J. Khan (2), B. W. Schäfer (1)

(1) Department of Oncology and Children's Research Center, University Children's Hospital Zurich, Steinwiesstrasse 75, 8032 Zürich , Switzerland, (2) Genetics Branch, National Cancer Institute, National Institutes of Health, Building 37, Room 2016B Bethesda, MD 20892, USA

In some cancers, aberrant transcription factors (TF) modulate gene expression to drive tumorigenesis. Targeting transcription in such malignancies represents a valuable strategy to interfere with the function of these oncogenic TFs and impair tumor development. This therapeutic approach is especially relevant for pediatric malignancies since they are commonly characterized by a low mutational burden and dependent on fusion TFs for survival. Fusion-positive rhabdomyosarcoma (FP-RMS) and Ewing sarcoma (ES) are two examples of this. Both tumors are driven by fusion TFs, PAX3-FOXO1 and EWS-FLI1, respectively, and are highly dependent on the fusion transcriptional signature.
One way of interfering with the transcriptional program of oncogenic fusion TFs is by targeting their epigenetic cofactors. Here, we explore the potential of the chromatin remodeler CHD4 as a novel therapeutic target in pediatric sarcomas. CHD4 (chromodomain-helicase-DNA binding protein 4) is an ATPase from the SNF2-like family able to move nucleosomes along the DNA and commonly associated with the NuRD (Nucleosome Remodeling and Deacetylase) complex. In FP-RMS and ES, CHD4 plays a crucial role as co-regulator of PAX3-FOXO1 and EWS-FLI1 signature and is essential for tumor cell survival. Mechanistically, in FP-RMS, we demonstrate that CHD4 co-localizes with PAX3-FOXO1 at super-enhancers (SE) together with BRD4. There,

CHD4 directly interacts with BRD4 and generates a chromatin architecture permissive for the binding of the fusion protein and of its co-factor BRD4. This allows the expression of the oncogenic program of PAX3-FOXO1 and permits tumor cell survival. In fact, CHD4 silencing leads to a decrease in acetylation and DNA accessibility at SE which correlates with a decrease in RNA Pol2 at promoters and a subsequent decrease in SE-driven gene expression. Consequently, CHD4 depletion causes tumor cell death in vitro and tumor regression in vivo. Our results demonstrate also that CHD4 regulates SE-driven gene expression in a NuRD-independent manner. In addition, we describe that ES is as well dependent on CHD4 most likely by a similar mechanism.

Notably, analysis of genome-wide cancer dependency databases identifies CHD4 as general novel cancer vulnerability with an effect comparable to the one observed for BRD4. Interestingly, CHD4 depletion in healthy human myoblasts and fibroblasts shows no effect on their proliferation.

Chromatin remodelers have been so far dismissed as drug targets. Here, we reveal for the first time not only a new role of CHD4 in the regulation of SE-driven oncogenic gene expression but also identify this chromatin remodeler as a general and possibly specific tumor susceptibility. Motivated by these results, we started developing the first-in-class CHD4 small molecule inhibitor through structure-based drug design and hope with this study to highlight the potential of chromatin remodelers as new drug targets for cancer therapy.

31

Transcriptome-Proteome Correlation in Human Hematopoietic Stem and Progenitor Cells

F. Meier-Abt* (4), S. Amon* (2), L. C. Gillet (2), S. Dimitrieva (3), A. P. A. Theocharides (1), M. G. Manz (1), R. Aebersold (2)
*These authors contributed equally to this work.

(1) Hematology, University and University Hospital Zurich, Zurich, Switzerland, (2) Department of Biology, Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland, (3) Functional Genomics Center Zurich, ETH Zurich and University of Zurich, Zurich, Switzerland, (4) Hematology, University and University Hospital Zurich, Zurich, Switzerland,

Introduction: Hematopoietic stem cells (HSCs) can self-renew and/or differentiate into various functionally divergent progenitor cell types, such as common myeloid progenitors (CMPs), megakaryocyte-erythrocyte progenitors (MEPs) or granulocyte-macrophage progenitors (GMPs). When the process of self-renewal and differentiation is altered, e.g. upon genetic or epigenetic changes in HSCs, abnormal (pre)leukemic stem cell subpopulations may form, eventually resulting in the onset of hematopoietic malignancies. To gain insight into the physiology and subsequent patho-physiology of self-renewal and differentiation, highly refined analyses of HSCs and downstream progenitor cells are needed. Whereas low numbers of cells are routinely analyzed by genomics and transcriptomics, corresponding proteomic analyses have so far not been possible due to methodological limitations.

Methods: We developed a new ultra-sensitive and robust quantitative proteomics technique based on data-independent acquisition mass spectrometry. We quantified the proteome of sets of 25,000 human hematopoietic stem/multipotent progenitor (HSC) and three committed progenitor cell subpopulations of the myeloid differentiation pathway (CMPs, MEPs, GMPs), isolated by fluorescence-activated cell sorting from five healthy donors. On average, 5,851 protein groups were identified per sample, and a subset of 4,131 stringently filtered protein groups was quantitatively compared across the 20 samples. The proteomic analyses were complemented by transcriptomic analyses.

Results: A comparison of proteomic and transcriptomic profiles of the respective cell types indicated hematopoietic stem/multipotent progenitor cell-specific divergent regulation of biochemical processes essential for maintaining stemness at the proteome rather than transcriptome level. Specifically, several telomerase maintenance proteins and quiescence-inducing isocitrate dehydrogenase proteins, both assumed to be essential for long-lived stem cells, were found to be upregulated in HSCs on the protein but not on the mRNA level when compared to myeloid progenitor cell subpopulations (CMPs, MEPs, GMPs).

Conclusions: The divergent mRNA/protein regulation of telomerase maintenance and quiescence-inducing isocitrate dehydrogenase proteins in HSCs illustrates the relevance of generating high quality proteomic data for well-defined cell subpopulations with the goal to identify biological processes that are insufficiently determined by genomic or transcriptomic analyses. The presented methodology is equally applicable to almost any rare cell type, including healthy and cancer stem cells or physiologically and pathologically infiltrating cell populations. It thus paves the way for proteomic profiling of relevant disease sample sub-fractions and, ultimately, should allow to find therapeutic targets in (pre)leukemic/cancer stem cells.

32

Role of IL-33/ST2 Signaling in Intestinal Tumorigenesis

V. Vu (1), M. H. Wasmer (1), E. Pastille (2), A. Adamczyk (2), C. Simillion (4), W. Hansen (2), S. Kasper (5), M. Schuler (5), B. Muggli (6), J. Buer (2), I. Zlobec (3), A. Westendorf (3), P. Krebs (3)

(1) University of Bern, Graduate School for Cellular and Biomedical Sciences, Bern, Switzerland, (2) Institute of Medical Microbiology, University Hospital Essen, Essen, Germany, (3) Institute of Pathology, University of Bern, Bern, Switzerland, (4) Department of Clinical Research, University of Bern, Bern, Switzerland, (5) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, University Duisburg-Essen, Essen, Germany, (6) Department of Visceral Surgery, Inselspital, Bern University Hospital, and University of Bern, Bern, Switzerland

Introduction: Interleukin-33 (IL-33) is a member of the IL-1 family of cytokines, mainly expressed by endothelial and epithelial cells where it is stored bound to the chromatin in the nucleus. It is believed to play a role through inhibiting transcription. Following tissue stress or damage, full-length IL-33 can release extracellularly as an alarmin and ind to its receptor, a heterodimeric receptor complex composed of an IL-1 accessory protein and IL-1RL1 (ST2). Engagement of ST2 activates several transcription factors such as NF-κB and AP-1, which are critical for transcription of inflammatory genes. Additionally, IL-33 has been involved in many inflammatory responses such as inflammatory bowel disease and virus-induced inflammation. However, the role IL-33 has to cancer pathogenesis is still elusive. In these projects, we will apply a translational approach to investigate the role of IL-33/ST2 signaling on myeloproliferative neoplasms and colorectal cancer.

Background: Regardless of significant improvements in prognosis and treatment in the last decade, colorectal cancer (CRC) remains to be one of the leading causes of cancer-related deaths. Genetic alterations in critical cellular pathways that associate with colon tumorigenesis have been identified. Evidence has shown chronic inflammation in the intestinal environment as a key promoter of this disease. For example, patients diagnosed with inflammatory bowel disease (IBD) have shown to have a higher risk of developing colorectal carcinomas. Mechanistically, recruitment of immune cells such as macrophages and neutrophils is triggered by the inflammation in the tumor tissue. These cells play a role in damaging DNA through their release of reactive oxygen species and nitrogen intermediates, thereby promoting the transformation of intestinal epithelial cells.

Previous Work: L-33 has shown to play an important role in the intestine, such as barrier permeability, healing and protection against helminth infection. Upon tissue stress or damage, IL-33 is released as an alarmin and binds to its receptor to increase inflammation. Until recently, little was known about the role IL-33 signaling played in CRC pathogenesis. Recent work in my group has shown data revealing a relationship in IL-33/ST2 signaling and CRC. We found that ST2 promotes accumulation of FOXP3 + regulatory T cells (T reg) in murine intestinal tumors, which correlated with increased tumor number and size. In addition, we have results showing that St2 -deficient mice are protected while IL33- deficient mice are susceptible to CRC, possibly reflecting the dual role of IL-33 as a transcriptional regulator or as an alarmin.

Objectives/Aims: For this project, we will elaborate the non-published data presented above by i) characterizing the tumor-infiltrating leukocytes that are dependent on IL-33/ST2 axis in CRC lesions and study how these cells contribute to tumorigenesis, while maintaining focus on the role of ST2 for T reg function, and ii) investigating the dual function of (nuclear versus soluble) IL-33 in CRC. I anticipate that the

planned translational investigations of this project will both increase our knowledge of the IL-33 mechanism and the role of IL-33/ST2 signaling for CRC pathogenesis. This will provide further insight on the potential of this pathway as a therapeutic target for intestinal cancer.

33

MHC II-dependent activation of regulatory T cells in the bone marrow of leukemia mice leads to immune evasion and disease progression

M. Hinterbrandner (1), A. F. Ochsenbein (2), C. Riether (1)

(1) Tumor Immunology, Department for BioMedical Research, University of Bern, Bern, Switzerland, (2) Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, Switzerland

Leukemia stem cells (LSCs) in the bone marrow (BM) are the origin of leukemia and resistant against conventional therapies and immune control. This resistance is partially mediated by protective mechanisms of the hematopoietic stem cell niche in the BM. In leukemia, the BM microenvironment changes dramatically with regulatory T cells (Tregs) accumulating. However, little is known how Tregs affect LSCs. We induced chronic myeloid leukemia (CML) in a murine model with BL/6 BCR-ABL-1 transduced LSKs (lineage-Sca-1^{+}c-kit^{+}) in FoxP3DTR-GFP mice. We investigated the frequency, origin, activation and proliferation capacity of BM Tregs in CML compared to naïve mice and analyzed the Treg-accumulation during disease progression.
BM Tregs in CML mice were mostly thymic-derived, activated and showed higher proliferation capacity compared to controls. Treg-depletion resulted in long-term survival in the majority of the mice. Importantly, Treg-depleted CML mice showed phenotypically decreased LSC numbers compared to controls (FACS-analysis) and also functionally by colony forming assays and secondary transplantation experiments. To investigate the possibility of an indirect preservation of LSCs via Tregs by inhibiting CD8 T cell mediated cell killing, we depleted both Tregs and CD8 T cells in leukemia-bearing mice. Parallel depletion restored LSC numbers, suggesting that Tregs protect LSCs from CD8-mediated elimination. To investigate the activation of Tregs, we induced CML derived from MHC-II-deficient LSCs since we observed high MHC II expression on LSCs. MHC II$^{-/-}$ CML developed significantly slower than control CML and showed the same phenotype as the Treg-depleted CML mice.
Our data indicate that thymic-derived, MHC-II-activated Tregs protect LSCs from elimination by cytotoxic CD8 T cells and promote leukemia development.

34

CRISPR/Cas9-based knock-out screening for long non-coding RNAs in lung cancer

T. Polidori, R. Esposito, C. Pulido, J. Bergadà Pijuan, R. Johnson

Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland

The human genome contains many thousands of long non-coding RNAs (lncRNAs), defined as large RNA transcripts longer than 200 nucleotides that do not code for proteins. The majority of lncRNA are still functionally uncharacterised, and may play roles in human disease. A small number of lncRNAs have been implicated in diseases and cancer.
A compelling question is how many of the remaining functionally uncharacterised 99% of lncRNAs may play similar disease roles.
Our hypothesis is first, that lncRNAs mediate and contribute to a cancer phenotype, and second, that such lncRNAs may be discovered using CRISPR-based pooled functional screening using cancer-related phenotypes. Thus, the aim of the project is to identify and characterise lncRNAs involved firstly in basic cancer phenotypes (proliferation) and progression, that could have a relevant significance and impact upon diseases.
In order to knock out lncRNAs, we use the validated approach of promoter deletion. We take advantage of two tools that we have developed for knockout of noncoding elements: a vector system called DECKO (Double Excision CRISPR Knockout) and CRISPETa, a bioinformatic pipeline for paired sgRNA design.
Using a high-throughput method that relies on CRISPR/Cas9, we propose to do a Loss of Function (LOF) screenings on lung cancer cell lines in order to find candidates that, when lost, lead to reduced proliferation. Moreover, in order to separate slow growing and fast growing populations of cells we aim to increase our power in the candidates identification by using a replication-dependent label such as CFSE (Carboxyfluorescencein succinimidyl ester) and FACS (Fluorescent-Activated Cell Sorting) to achieve their phisical separation.
Such candidates would be therefore evaluated individually for their role in cell proliferation with different methods and in multiple lung cancer cell lines.

In conclusion, the increasing understanding of the mechanisms by which lncRNAs function, will allow the identification of the best targets for diagnostics and therapies.

35

Peripheral serotonin dampens tumor-specific immune response via upregulation of PD-1/PD-L1 expression

M. A. Schneider (1), M. M. Beffinger (2), S. Pantelyushin (2), U. Ungethüm (1), R. Graf (1), J. Vom Berg (2), A. Gupta (1), P. A. Clavien (1)

(1) HPB Research Laboratory, Department of Surgery, University Hospital Zurich, Switzerland, (2) Transgenic Core Facility, Institute of Laboratory Animal Science, University of Zurich, Switzerland

Background: Serotonin (5-HT), a well-known neurotransmitter, is biochemically derived of the amino acid tryptophan through a multistep pathway including the rate-limiting enzyme tryptophan hydroxylase (TPH). Two different genes encode TPH: TPH1 is responsible for peripheral 5-HT supply, while 5-HT production in neurons exclusively relies on TPH2. Approximately 95% of 5-HT is produced by TPH1 in enterochromaffin cells of the intestinal mucosa. Immediately after its synthesis and release, serotonin is taken up by thrombocytes, which store it in dense granules and release it upon activation. Peripheral 5-HT has pleiotropic effects on a multitude of peripheral (patho-) physiological processes including coagulation, vasotonus regulation, bone-metabolism, regulation of energy-balance, liver-regeneration, tissue-repair after ischemia/reperfusion and development of steatohepatitis. Furthermore, 5-HT has also been implicated to promote cancer cell proliferation and tumor growth. However, the influence of 5-HT on the tumor microenvironment and tumor-specific immune responses remains elusive. Therefore, using peripheral serotonin deficient mice (Tph1$^{-/-}$ mice), we explored the effects of 5-HT on the interaction between immune and cancer cells within the tumor microenvironment.

Methods: Wt (C57BL/6) and Tph1$^{-/-}$ mice were injected with the syngeneic cancer cells lines MC38 (colorectal carcinoma) or Panc02 (pancreatic carcinoma). Tumors cells were implanted subcutaneously (s.c.) or orthotopically and tumor growth was measured by calipometer every second day or via intraabdominal MRI. At the endpoint, tumors were collected for immunohistochemistry (IHC), qPCR and FACS analysis. Tumor-associated immune cells were quantified using IHC and FACS.

Results: Compared to Wt mice, Tph1$^{-/-}$ mice had significantly reduced tumor burden after both orthotopic and s.c. injection of MC38 or Panc02 cells. Within tumors of Tph1$^{-/-}$ mice, we noted enhanced accumulation of functional CD8^{+} T-cells. Depletion of CD8^{+} T-cells resulted in increased and equalized tumor growth, confirming that cytotoxic T-cells impair tumor growth in Tph1$^{-/-}$ mice. qPCR-based screening of parameters influencing the tumor microenvironment revealed reduced expression of PD-L1 within tumors of Tph1$^{-/-}$ mice, suggesting serotonin may upregulate PD-L1 expression. Treatment of murine and human cancer cells with serotonin in-vitro enhanced expression of PD-L1 on cancer cells, which could partly be prevented by addition of a SERT blocker. Additionally, increased levels of PD-1 on tumor-infiltrating T-cells was observed in Wt animals. Indeed, 5-HT stimulation of T-cells in-vitro lead to PD-1 expression in resting state and upon activation, while other markers of T-cell activation & exhaustion were not altered.
Pharmacological inhibition of platelet activation & 5-HT release using Prasugrel or inhibition of 5-HT uptake into platelets through the SERT inhibitor fluoxetine decreased tumor growth in Wt mice similar to Tph1$^{-/-}$ mice. On further analyses, we observed increased influx of CD8^{+} T-cells and reduced expression of PD-L1 within tumors of WT that were treated with Prasugrel or fluoxetine. Vice versa, reconstitution of 5-HT levels in Tph1$^{-/-}$ mice promoted tumor growth, reduced CD8^{+} T-cell accumulation and enhanced PD-L1 expression within tumors. Subsequently, s.c. injection of MC38-PD-L1$^{-/-}$ cells in Wt and Tph1$^{-/-}$ mice resulted in similar tumor growth, confirming the link between 5-HT-induced PD-L1 expression, CD8^{+} T-cell accumulation and tumor growth.

Conclusions: Peripheral 5-HT enhances upregulation of PD-1/PD-L1 in the tumor-microenvironment, thereby skewing T-cells towards exhaustion. Pharmacological inhibition of SERT reduces serotonin levels within tumors and decreases tumor growth. Therefore, combination of serotonin-blockade with checkpoint-inhibitor therapy might augment effects of current immunotherapy regimens and prove beneficial in long-term control of tumors.

36

Tumor heterogeneity during the progression of metastatic breast cancer

S. Tiede (1), R. K. R. Kalathur (1, 2), L. Von Allmen (1), B. Szczerba (1), T. Vlajnic (3), N. Aceto (1), G. Christofori (1)

(1) Department of Biomedicine, University of Basel, Basel, Switzerland, (2) Swiss Institute of Bioinformatics, Basel, Switzerland (3) Institute of Pathology, University Hospital Basel, Basel, Switzerland

Every year, over 450,000 women lose their lives to breast cancer, mostly due to metastasis. Over the past decades, reasonable progress has been made in the understanding of breast cancer biology and the treatment of the primary tumor. However, the process of tumor progression from a premalignant disease to invasive carcinoma and metastatic outgrowth is still poorly understood and the overall survival rates for metastatic disease remain poor. In particular, the molecular and functional contribution of tumor heterogeneity or, in other words, of multiple cancer cell subclones on tumorigenesis and particularly metastasis formation remains largely elusive [1].
This project aims to assess clonal expansion and tumor heterogeneity during tumor progression using the MMTV-PyMT mouse model of metastatic breast cancer expressing the Confetti lineage reporter [2,3]. For this purpose, mammary epithelial cells of female mice have been induced to express one of the four Confetti reporter fluorescent proteins. The outgrowth of clonal cell populations has been analyzed when the maximum tumor volume comprising all stages (normal, hyperplasia, adenoma, carcinoma, pulmonary metastases) had been reached. The Confetti lineage tracing system visualizes the emergence of clonal populations of various stages in the primary tumor and medium mono- and polychromatic metastatic lesions in the lung. Zonal patches of clonal populations in the primary tumor as well as metastases were isolated by Laser Capture Microdissection and the samples obtained were subjected to RNA sequencing [4,5]. Comparative analyses of gene expression profiles of various clonal primary tumor stages and metastatic lesions indicate a substantial level of heterogeneity across the stages and even within a stage of tumor progression. Further bioinformatics analysis will contribute to the understanding of clonal heterogeneity during breast cancer progression to finally develop new targeted therapies for this presently incurable disease.

1. Koren, S. and M. Bentires-Alj, Breast Tumor Heterogeneity: Source of Fitness, Hurdle for Therapy. Mol Cell, 2015. 60(4): p. 537-46.

2. Livet, J., et al., Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. Nature, 2007. 450(7166): p. 56-62.

3. Lin, E.Y., et al., Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. Am J Pathol, 2003. 163(5): p. 2113-26.

4. Chen, J., et al., Spatial transcriptomic analysis of cryosectioned tissue samples with Geo-seq. Nature protocols, 2017. 12(3).

5. Picelli, S., et al., Full-length RNA-seq from single cells using Smart-seq2. Nature protocols, 2014. 9(1).

37

Protein stability screen to identify compounds destabilizing EWS-FLI1 in Ewing sarcoma

G. Pedot, F. Niggli, B. Schaefer

Oncology department, University Children's Hospital, Zurich

Ewing sarcoma is an aggressive pediatric bone and soft tissue tumor driven by the expression of a fusion oncoprotein named EWS-FLI1, which acts as an oncogenic transcription factor. Tumor cells are strictly dependent on continuous expression of the fusion protein, since downregulation of EWS-FLI1 inhibits tumor growth.
Therefore, interference with the fusion oncoprotein turnover is critical for the modulation of tumor cell proliferation and survival. We demonstrated previously that EWS-FLI1 is predominantly a proteasomal substrate with high turnover mediated by poly-ubiquitination at one specific lysine. This study provided novel insights into the crucial importance of targeting EWS-FLI1 stability as novel strategy for the treatment of Ewing sarcoma.
Hence, we aimed at identifying compounds that destabilize EWS-FLI1 with subsequent reduction of tumor cell growth by performing a screen of 2'486 FDA-Approved drugs and 204 novel targeted compounds in a Ewing sarcoma reporter cell line. To this, we adopted a Global Protein Stability approach as novel read-out (Global protein stability profiling in mammalian cells, Science, 2008), which relies on a reporter construct expressing two fluorescent dyes from one mRNA to monitor changes in stability of EWS-FLI1 by high-throughput flow cytometry. This drug screen identified two main enriched classes of inhibitors that significantly destabilize EWS-FLI1. Validation of these

results and characterization of their mechanism of action is currently under way.
We conclude that the study of EWS-FLI1 turnover represents a novel approach to identify new effective drugs that can be used as monotherapy or in combination with other drugs as novel treatment opportunities in Ewing sarcoma.

38

Cytotoxicity of nimbolide towards multidrug-resistant tumor cells and hypersensitivity via cellular metabolic modulation

N. Mahmoud (1), M. Saeed (1), Y. Sugimoto (2), S. Klauck (3), H. Greten (4), T. Efferth (1)

(1) Department of Pharmaceutical Biology, Johannes Gutenberg University, Mainz, Germany, (2) Division of Chemotherapy, Faculty of Pharmacy, Keio University, Tokyo, Japan, (3) Division of Cancer Genome Research, German Cancer Research Center, German Cancer Consortium, National Center for Tumor Diseases, Heidelberg, Germany, (4) Heidelberg School of Chinese Medicine, Heidelberg

Nimbolide is considered a promising natural product in cancer prevention and treatment. However, it is not known yet, whether the different mechanisms of multidrug resistance (MDR) influence its anticancer activity. In this study, well-known MDR mechanisms (ABCB1, ABCG2, ABCB5, TP53, EGFR) were evaluated against nimbolide. The P-glycoprotein (ABCB1/MDR1)-overexpressing CEM/ADR5000 cell line displayed remarkable hypersensitivity to nimbolide, which was mediated through upregulation of the tumor suppressor, PTEN, and its downstream components resulted in significant downregulation in ABCB1/MDR1 mRNA and P-glycoprotein. In addition, nimbolide targeted essential cellular metabolic-regulating elements including HIF1α, FoxO1, MYC and reactive oxygen species. The expression of breast cancer resistance protein (BCRP), as well as epidermal growth factor receptor (EGFR) and mutant tumor suppressor TP53, did not correlate to nimbolide's activity. Furthermore, this we looked for other molecular determinants that might determine tumor cellular response towards nimbolide. COMPARE and hierarchical cluster analyses of transcriptome-wide microarray-based mRNA expressions of the NCI 60 cell line panel were performed, and a set of 40 genes from different functional groups was identified. The data suggested NF-κB as a master regulator of nimbolide's activity. Interestingly, HIF1α was determined by COMPARE analysis to mediate sensitivity to nimbolide, which would be of great benefit in targeted therapy.

39

Dendritic cells predict the responsiveness of PD-L1 blockade in cancer

M. Mayoux (1), A. Roller (2), V. Pulko (1), S. Chen (1), M. Fransen (3), R. Buser (1), M. Kowanetz (4), K. Rommel (1), I. Matos (1), S. Colombetti (1), A. Belousov (5), V. Karanikas (1), F. Ossendorp (3), P. Hegde (4), D. Chen (6), P. Umana (1), M. Perro (1), C. Klein (1), W. Xu (1)

(1) Cancer Immunotherapy Discovery, Pharmaceutical Research and Early Development, Roche Innovation Center Zurich, Schlieren, Switzerland, (2) Pharmaceutical Sciences, Biomarkers, Bioinformatics and Omics, Pharmaceutical Research and Early Development, Roche Innovation Center Basel, Basel, Switzerland, (3) Dept. of Immunohematology and Bloodtransfusion, Leiden University Medical Center, Leiden, The Netherlands, (4) Oncology Biomarker Development, Genentech, Inc., 1 DNA Way, South San Francisco, California 94080, USA, (5) Pharmaceutical Sciences, Biomarkers, Bioinformatics and Omics, Pharmaceutical Research and Early Development, Roche Innovation Center Munich, Penzberg, Germany, (6) Product Development, Genentech, Inc., 1 DNA Way, South San Francisco, California 94080, USA

PD-L1/PD-1 inhibitors have demonstrated therapeutic efficacy across a range of human cancers. However, it remains incompletely understood which patients may or may not respond to PD-1/PD-L1 blockade. Extending this benefit to a greater number of patients will require a better understanding of how these therapies affect anti-cancer immunity. Although blocking PD-L1/PD-1 axis signaling is typically associated with reinvigoration of tumor-infiltrating PD-1$^+$ T cells, we demonstrate an important role of the dendritic cell (DC) in response to PD-L1 blockade. Indeed, we discover that DC gene signature in patients with renal cell carcinoma and non-small cell lung cancer is strongly associated with improved overall survival (Phase I/II clinical studies of atezolizumab). We sought the mechanism understanding of PD-L1 blockade on DCs. Using human and mouse monocyte-derived DCs for T cell priming and a protein interaction assay for validation; we showed that blocking PD-L1 relieves B7.1 sequestration in cis by PD-L1 and renders DCs more capable of providing a costimulatory signal via the B7.1/CD28 interaction leading to enhanced T cell priming. These data suggest that PD-L1 blockade reinvigorates DC function to generate potent anti-cancer T cell immunity. This is an important finding to predicting patients for maximum benefit of atezolizumab therapy.

40

Triple therapy targeting metastatic niche-evoked drug resistance in triple-negative breast cancer

R. Amante (1), D. De Silva (1), M. Ackerknecht (1), M. M. Coissieux (1), V. Romanet (2), A. Sethi (1), B. T. Preca (1), B. Hamelin (1), S. Piscuoglio (4), C. K. Y. Ng (4), M. M. Mohseni (3), M. Bentires-Alj (1)

(1) Department of Biomedicine University of Basel, Switzerland, (2) Novartis Institutes for Biomedical Research, Basel, (3) Novartis Institutes for Biomedical Research, Cambridge, MA, USA, (4) Institute of Pathology, University and University Hospital Basel

Resistance to PI3K inhibition is often driven by activation of receptor tyrosine kinases (RTKs). Given that the protein tyrosine-phosphatase SHP2 activates oncogenic pathways downstream of most RTKs, we assessed the effect of co-targeting both PI3K and SHP2 in preclinical models of metastatic triple negative breast cancer (TNBC). Dual PI3K/SHP2 inhibition induced apoptosis, decreased primary tumor growth synergistically, blocked the formation of lung metastases, and increased overall survival in both neoadjuvant and adjuvant settings. Mechanistically, SHP2 inhibition resulted in the activation of PI3K signaling and the sensitization of TNBC cells to inhibition of this pathway. Lung metastases were rare after PI3K/SHP2 inhibition but more liver metastases were recorded, suggesting different resistance mechanisms in the lung and the liver niches. In contrast, triple blockade of PI3K, SHP2 and VEGFR/PDGFR decreased liver metastases and increased overall survival of the animals. This provides a rationale for co-targeting these pathways in metastatic TNBC.

41

Long non-coding RNA E2F4as promotes tumor progression and predicts patient prognosis in human ovarian cancer

H. J. Kim (1), S. A. Park (1), Y. T. Kim (2), T. H. Heo (1)

(1) Integrated Research Institute of Pharmaceutical Sciences, College of Pharmacy, (2) Institute of Women's Life Medical Science, Department of Obstetrics and Gynecology, Yonsei University College of Medicine

Purpose: The functions of many long non-coding RNAs (lncRNAs) in human cancers remain to be clarified. Induction of E2F4as (antisense) by Wnt signaling may contribute to carcinogenesis by reducing levels of the E2F4as cell cycle repressor in colorectal cancer. Disruption of Wnt signaling is common in ovarian cancer. Despite the proposed models of E2F4as function, the significance of E2F4as RNA remains unclear in cancer. In this study, we examined the expression level of E2F4as in the serum of ovarian cancer patients and the functional role of E2F4as in ovarian cancer.

Methods: The serum samples were obtained from 116 pathological diagnosed ovarian cancer patients and 39 normal age-matched women. The expression of E2F4as was measured by real time RT-PCR. To investigate the role of E2F4as in cell proliferation, invasion, and migration, E2F4as expression in ovarian cancer cells was knocked down using RNA interference.

Results: The expression of E2F4as was significantly higher in the serum of ovarian cancer patients than in control patients ($P < 0.05$). E2F4as siRNA in SKOV3 cells decreased cell proliferation, invasion, and migration. Moreover, Knockdown of E2F4as decreased the expression of epithelial-mesenchymal transition (EMT), which are important for cell motility and metastasis. Mechanistic investigation revealed that Notch1, Hes1 and p300 proteins could be inhibited by E2F4as depletion.

Conclusions: These findings highlight the clinical significance of E2F4as in predicting the prognosis of ovarian cancer patients and suggest its potential in promoting tumor aggressiveness by regulation of the Notch signaling pathway and EMT-related mechanisms.

42

A selective inhibitor of the Polo-box domain of Polo-like kinase 1 identified by virtual screening

S. Abdelfatah (1), A. Berg (2), M. B. Böckers (1), T. Efferth (1)

(1) Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz 55128, Germany, (2) Leipzig University, Institute of Organic Chemistry Johannisallee 29, 04103 Leipzig, Germany

Polo-like kinase 1 (PLK1), a member of the Polo-like kinase family, plays an important regulatory role in mitosis and cell cycle progression. PLK1 overexpression is correlated with tumourigenesis and poor prognosis in cancer patients. Therefore, the identification of novel compounds that inhibit PLK1 would provide attractive therapeutic approaches. Although some PLK1 kinase inhibitors have been developed, their application has been limited by off-target effects. PLK1 contains a regulatory domain named the Polo-box domain (PBD), which is characteristic only for the Polo-like kinase family. This domain represents an alternative therapeutic target with higher selectivity for PLK1.

In this study, we applied in silico virtual drug screening, fluorescence polarization and microscale thermophoresis to identify new scaffolds targeting the PBD of PLK1. One compound, 3-{[(1R,9S)-3-(naphthalen-2-yl)-6-oxo-7,11-diazatricyclo[7.3.1.0^{2,7}]trideca-2,4-dien-11-yl]methyl}benzonitrile (designated compound (1)),out of a total of 30,793 natural product derivatives,inhibited thePLK1 PBD with high selectivity (IC50: 17.9 ± 0.5 μM). This compound inhibited the growth of cultured leukaemia cells (CCRF-CEM and CEM/ADR5000) and arrested the cell cycle in the G2/M phase, which is characteristic for PLK1 inhibitors. Immunofluorescence analyses showed that treatment with compound (1) disrupted spindle formation due to the aberrant localization of PLK1 during the mitotic process, leading to G2/M arrest and ultimately cell death.

In conclusion, compound (1) is a selective PLK1 inhibitor that inhibits cancer cell growth. It represents a chemical scaffold for the future synthesis of new selective PLK1 inhibitors for cancer therapy.

43

L1CAM defines an ovarian cancer stem cell population

N. Terraneo (1), F. Jacob (2), C. Peitzsch (3), A. Dubrovska (3), V. Heinzelmann-Schwarz (2), M. Béhé (1), R. Schibli (4), J. Grünberg (1)

(1) Center for Radiopharmaceutical Sciences ETH-PSI-USZ, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland, (2) Department of Biomedicine, University of Basel, 4031 Basel, Switzerland, (3) OncoRay-National Center for Radiation Research in Oncology, 01309 Dresden, Germany, (4) Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland

Many solid tumors, including ovarian cancer (OC), contain small populations of tumor-initiating cells, also known as cancer stem cells (CSCs). Overlapping cell populations displaying expression of different cell surface markers such as CD24, CD44, CD133 and aldehyde dehydrogenase (ALDH) enzymatic activity have been characterized as ovarian CSCs [1]. These cells usually show high resistance against conventional cancer therapies and seem to be involved in metastasis and tumor relapse. It is now proved that only CSCs have the ability to proliferate under non-differentiating and non-adherent conditions, forming three-dimensional multicellular tumor spheroids which are very aggressive in growth and show reduced response to chemotherapeutic drugs in vitro.

L1 cell adhesion molecule (L1CAM) is a highly glycosylated type I transmembrane protein that plays a role in the development of the nervous system and in human cancer. In cancer, L1CAM expression induces motile and an invasive phenotype, supporting aggressive tumor growth, metastasis and chemoresistance. Recently, it was shown that L1CAM is a CSC-specific marker in glioblastoma [2]. However, whether this molecule plays a role in ovarian CSCs is so far unknown.

Based on these findings we focus our research to elucidate the biological role of L1CAM in ovarian CSCs. Specific populations of cells expressing L1CAM alone or in combination with CD133 were isolated by fluorescence-activated cell sorting (FACS) from various established OC cell lines. Plating efficiency and radioresistance were assessed by colony-forming assay. The spherogenic capacity of the different cell populations was analyzed. The results show that L1CAM+/CD133+ population has higher spherogenic and clonogenic properties in comparison to L1CAM-/CD133- cell population. Moreover, L1CAM+/CD133+ cell population retains the highest clonogenic capacity after irradiation, indicating high radioresistance of these cells. Interestingly, L1CAM seems to be responsible for radioresistance in this cell

population. The double positive cells also show higher tumor take, self-renewal and faster tumor growth in nude mice when compared with the other cell populations. Additionally, the expression of some CSC-specific genes seems to be up-regulated in L1CAM+/CD133+ population. These results indicate that L1CAM, in combination with CD133, might define a specific population of ovarian tumor-initiating cells.

1. Silva, I. A. et al. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. Cancer Res. 71, 3991–4001 (2011).

2. Bao, S. et al. Targeting cancer stem cells through L1CAM suppresses glioma growth. Cancer Res. 68, 6043–6048 (2008

44

Evaluation of bivalent grpr radioligands for targeting prostate cancer

N. Romantini (1), S. Dobitz (2), M. D. Alam (1), M. Spillmann (4), R. Schibli (1), X. Deupi (3), H. Wennemers (2), P. Berger (4), M. Béhé (1)

(1) Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Villigen, (2) Laboratory of Organic Chemistry, ETH, Zurich, (3) Laboratory of Biomolecular Research, Paul Scherrer Institute, Villigen, (4) Laboratory of Nanoscale Biology, Paul Scherrer Institute, Villigen

Targeted radiotherapy (TRT) aims at the destruction of tumor tissue by radioactivity specifically delivered to the neoplastic lesion. Since the gastrin-releasing peptide receptor (GRPR) is overexpressed on prostate cancer cells, it is an ideal target for TRT [1]. In this project, ^{177}Lu-labeled bivalent peptides are evaluated as high affinity ligands for GRPR. Two agonistic recognition motifs are fused on a rigid oligoproline backbone in different distances from each other (10, 20 or 30 Å), forming the three compounds BBN-10, BBN-20 and BBN-30 [2]. A monovalent oligoproline-backbone-based compound (BBN-00) as well as the agonist AMBA [3] that is well known from literature served as references.

We determined the receptor-mediated cellular uptake of all compounds as well as the biodistribution of BBN-20 and AMBA in tumor-bearing mice. Moreover, we explored the influence of bivalent ligands on receptor signaling using luciferase complementation and BRET assays for better understanding of the binding and internalization mechanism of bivalent ligands. Furthermore, we included computational modelling of receptor-ligand interaction to confirm our experimental findings.

All bivalent compounds showed significantly higher cellular uptake (BBN-10: 29.2 ± 2.3%, BBN-20: 28.2 ± 2.5% and BBN-30: 20.2 ± 3.5%) than the monovalent reference BBN-00 (7.0 ± 1.4%) supporting the idea to use bivalency as a tool to increase receptor affinity. Moreover, the cellular uptake was clearly distance-dependent since BBN-30 showed significantly lower uptake than BBN-10 and BBN-20. None of the oligoproline-based ligands outperformed the literature reference AMBA (43.5 ± 3.5%) indicating a sterical hindrance of the backbone. However, biodistribution of BBN-20 resulted in a 2.7-fold increase of tumor uptake compared to the literature reference AMBA. These results suggest that bivalency increases specificity for the tumor in vivo.

References:

1. Reubi, J. C. et al. Bombesin receptor subtypes in human cancers: detection with the universal radioligand (125)I-[D-TYR(6), beta-ALA(11), PHE(13), NLE(14)] bombesin(6-14). Clin. Cancer Res. 2002; 8: 1139–46

2. Kroll, C. et al. Hybrid bombesin analogues: Combining an agonist and an antagonist in defined distances for optimized tumor targeting. J. Am. Chem. Soc. 2013; 135: 16793–16796

3. Lantry, L. E. et al. 177Lu-AMBA: Synthesis and characterization of a selective 177Lu-labeled GRP-R agonist for systemic radiotherapy of prostate cancer. J. Nucl. Med. 2006; 47: 1144–1152

45

Opposing effects of cancer type-specific SPOP mutations on BET protein degradation and sensitivity to BET inhibitors

G. El Tekle (1), H. Janouskova (1), E. Bellini (2), N. Udeshi (3), A. Rinaldi (1), A. Ulbricht (9), T. Bernasocchi (1), G. Civenni (1), M. Losa (1), T. Svinkina (3), C. M. Bielski (3), GV. Kryukov (3), L. Casciono (1), S. Napoli (1), R. I. Enchev (9), D. G. Mutch (4), M. E. Carney (5), A. Berchuck (6), B.J.N. Winterhoff (7), R. R. Broaddus (8), P. Schraml (2), H. Moch (2), F. Bertoni (1), C. V. Catapano (1), M. Peter (9), S. A. Carr (3), L. A. Garraway (3), P. J. Wild (2), J. P. P. Theurillat (1)

(1) Institute of Oncology Research, Bellinzona, (2) Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, (3) Broad Institute, MIT & Harvard, Cambridge, MA, (4) Division of Gynecologic Oncology, Washington University, St. Louis, (5) Department of Obstetrics, Gynecology and Women's Health, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, (6) Division of Gynecologic Oncology, Duke Cancer Center, Durham, (7) Division of Gynecologic Oncology, University of Minnesota, Minneapolis, (8) Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, (9) Department of Biochemistry, ETH, Zurich

It is generally assumed that recurrent mutations within a given cancer driver gene elicit similar drug responses. Cancer genome studies have identified recurrent but divergent missense mutations affecting the substrate-recognition domain of the ubiquitin ligase adaptor SPOP in endometrial and prostate cancers. The therapeutic implications of these mutations remain incompletely understood. Here we analyzed changes in the ubiquitin landscape induced by endometrial cancer–associated SPOP mutations and identified BRD2, BRD3 and BRD4 proteins (BETs) as SPOP–CUL3 substrates that are preferentially degraded by endometrial cancer–associated SPOP mutants. The resulting reduction of BET protein levels sensitized cancer cells to BET inhibitors. Conversely, prostate cancer–specific SPOP mutations resulted in impaired degradation of BETs, promoting their resistance to pharmacologic inhibition. These results uncover an oncogenomics paradox, whereby mutations mapping to the same domain evoke opposing drug susceptibilities. Specifically, we provide a molecular rationale for the use of BET inhibitors to treat patients with endometrial but not prostate cancer who harbor SPOP mutations.

46

Identification of physiologically relevant EWS-FLI1 target genes in Ewing's sarcoma via CRISPRa screening

V. Saratov, Q.A. Ngo, G. Pedot, B.W. Schäfer, F.K. Niggli

Oncology department, University Children's Hospital, Zurich

Ewing's sarcoma is an aggressive pediatric bone and soft tissue cancer and the second most common primary bone tumor. A pathognomonic chromosomal translocation t(11;22) results in expression of EWS-FLI1, a fusion protein present in 85% of cases, thus presenting an excellent therapeutic target. However, due to its nature as transcription factor, directly disrupting EWS-FLI1 activity is challenging. Alternative strategies include targeting EWS-FLI1 synthesis or downstream target genes, activated or repressed by the fusion protein.

In this study, we aim to identify and rank EWS-FLI1 target genes essential for cancer cell survival providing potential novel therapeutic targets. To this end, we generated Ewing sarcoma cell lines expressing a CRISPRa (activation) system. To provide homogenous genetic background, we pre-selected 21/180 CRISPRa single clones. Importantly, we found robust expression of CD44, a surface marker absent on the surface of used Ewing cells, in some of these clones, which were additionally characterized for homogeneity of the population. This system allows us to functionally interrogate EWS-FLI1 repressed target genes both in vitro and in vivo.

To this, we are generating a sgRNA-library covering EWS-FLI1-repressed target genes, which are bioinformatically selected from publically available EWS-FLI1 silencing RNA-Seq datasets. Potential hits from the screen will be validated and ranked by their effect on tumor viability after activation by CRISPRa.

Taken together, the establishment of a gene expression activating system will allow us to test EWS-FLI1 target gene library to identify potential new therapy options and add to our current understanding of the biology of the fusion protein.

47

The tumor suppressive TGF-β/SMAD1/S1PR2 signaling axis is recurrently inactivated in diffuse large B-cell lymphoma

A. Stelling (1), C. T. Wu (1), H. Hashwah (1), K. Bertram (1), A. Tzankov (2), A. Müller (1)

(1) Institute of Molecular Cancer Research, University of Zurich, Switzerland, (2) Institute of Pathology, Basel University Hospital, Basel, Switzerland

The sphingosine-1-phosphate receptor S1PR2 and its downstream signaling pathway are commonly silenced in diffuse large B-cell lymphoma (DLBCL), either by mutational inactivation or through negative regulation by the oncogenic transcription factor FOXP1.
We have examined the upstream regulators of S1PR2 expression and have newly identified the TGF-ß/TGF-ßR2/SMAD1 axis as critically involved in S1PR2 transcriptional activation. Phosphorylated SMAD1 directly binds to regulatory elements in the S1PR2 locus as assessed by chromatin immunoprecipitation, and the CRISPR-mediated genomic editing of S1PR2, SMAD1 or TGFBR2 in DLBCL cell lines renders cells unresponsive to TGF-ß-induced apoptosis. DLBCL clones lacking any one of the three factors have a clear growth advantage in vitro, as well as in subcutaneous xenotransplantation models, and in a novel model of orthotopic growth of DLBCL cells in the spleens and bone marrow of MISTRG mice expressing various human cytokines. The loss of S1pr2 induces hyper-proliferation of the germinal center B-cell compartment of immunized mice and accelerates MYC-driven lymphomagenesis in spontaneous and serial transplantation models. The specific loss of Tgfbr2 in murine GC B-cells phenocopies the effects of S1pr2 loss on GC B-cell hyper-proliferation. Finally, we were able to show that SMAD1 expression is aberrantly downregulated in >85% of analyzed DLBCL patients.
The results uncovered an important novel tumor suppressive function of the TGF-ß/TGF-ßR2/SMAD1/S1PR2 axis in DLBCL, and show that DLBCL cells have evolved to inactivate the pathway at the level of SMAD1 expression. Currently we are examining the epigenetic silencing of SMAD1 in DLBCL via bisulfite sequencing of patient samples and DLBCL cell lines. Furthermore we are investigating the possibility of targeting epigenetically silenced SMAD1 with drugs in various newly established mouse models of DLBCL.

48

Anti-tumourigenic effects of the iron chelator ciclopirox in HPV-positive cancer cells

A. L. Herrmann (1), J. Braun (1), B. Kuhn (2), J. Krijgsveld (2), K. Hoppe-Seyler (1), F. Hoppe-Seyler (1)

(1) Molecular Therapy of Virus-Associated Cancers, German Cancer Research Center, Heidelberg, (2) Proteomics of Stem Cells and Cancer, German Cancer Research Center, Heidelberg

Human Papillomaviruses (HPVs) are major human carcinogens and the causative agents for a large percentage of anogenital and head and neck cancers. HPV-positive cancer cells rely on the expression of the viral oncogenes E6 and E7 to maintain their malignant phenotype. Repression of these oncogenes leads to rapid induction of senescence, which makes E6 and E7 attractive therapeutic targets. By investigating the crosstalk between HPVs and the cellular iron metabolism, we recently discovered that iron chelators efficiently suppress E6/E7 expression. For further analyses we focused on the iron chelator ciclopirox (CPX), which is used since decades for the topical treatment of fungal infections of the skin and mucosa. We found that CPX strongly downregulates E6 and E7 on both mRNA and protein level. Phenotypically, CPX efficiently suppresses cell growth of HPV-positive tumour cells in both 2D- and 3D (spheroid)-cell culture. Treatment with CPX induces a proliferative stop in the G1 phase of the cell cycle and a senescent phenotype. Moreover, after prolonged treatment, CPX leads to apoptosis of HPV-positive cancer cells. Next to its anti-oncogenic potential as a single therapeutic agent, we found that CPX can enhance the effects of radio- and chemotherapy on HPV-positive cancer cells.

In order to elucidate the mechanism behind the anti-proliferative effect of CPX, we conducted a comprehensive proteomics screen in CPX-treated HPV-positive cancer cells. Several interesting cancer-linked candidate genes and pathways, which are regulated by CPX, could be identified and successfully validated. One of the highest ranked differentially regulated genes in the screen was Notch-1. The Notch signalling pathway has a tumour suppressive role in epithelial tissues and upregulation would thus be desirable for a potential anti-cancer drug. Indeed, we obtained experimental evidence revealing a stimulation of the Notch signalling cascade by CPX in HPV-positive tumour cells.

Taken together, we identified the iron chelator ciclopirox as a potential new treatment option for HPV-positive cancers. Ciclopirox (I) efficiently blocks viral E6/E7 oncogene expression, (II) activates tumour suppressive signalling pathways, (III) exerts profound anti-tumourigenic effects in HPV-positive cancer cells and (IV) shows a favourable combinatorial effect with radio- and chemotherapy. Moreover, since the systemic application of iron chelators can be associated with strong side effects, the topical administration route of CPX may offer the possibility to achieve high local drug concentrations in HPV-induced neoplasias. Thus, CPX may serve as a basis for the development of innovative therapeutic strategies for the treatment of HPV-positive cancers.

49

Dissecting the role of autophagy in normal and malignant B cells

M. Arambasic, U. Novak

Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland

B-lymphocytes development from hematopoietic stem cells starts in the bone marrow and continues in peripheral lymphoid organs in the adult. Maturation steps of most B-cells are linked to a highly specialized microenvironment within peripheral lymphoid organs named germinal center (GC). In GCs, B-cells undergo clonal expansion and their immunoglobulin genes are diversified by somatic hypermutation and class-switch recombination. These two physiological DNA modifications may give rice to genetic abnormalities that could lead to lymphomas. The majority of B-cell lymphomas derive from germinal center experienced B cells.
We currently focus on the role of autophagy in two types of non-Hodgkin B-cell lymphomas: diffuse large B-cell lymphomas (DLBCL) and Burkitt lymphomas. We have been interested whether DLBCL and Burkitt cell lines are autophagy-competent and whether they differ in their response to treatments with autophagy inhibitors and inducers.

50

Genetic dissection of the miR-200-Zeb1 axis reveals its importance in tumor differentiation and invasion

A. C. Title (2), S. J. Hong (1), N. D. Pires (2), L. Hasenöhrl (2), S. Godbersen (2), N. Stokar-Regenscheit (3), D. P. Bartel (1), M. Stoffel (2)

(1) Howard Hughes Medical Institute and Program in Molecular Medicine, University of Massachusets Medical School, Worcester, (2) Institute of Molecular Health Sciences, Department of Biology, Swiss Federal Institute of Technology Zurich, Zurich, (3) Institute of Animal Pathology (COMPATH), University of Bern, Bern

The epithelial-to-mesenchymal transition (EMT) is an important mechanism for cancer progression and metastasis. Numerous in vitro and tumor-profiling studies point to the miR-200-Zeb1 axis as crucial in regulating this process, yet in vivo studies involving its regulation within a physiological context are lacking. Here, we show that miR-200 ablation in the Rip-Tag2 insulinoma mouse model induces beta-cell dedifferentiation, initiates an EMT expression program, and promotes tumor invasion. Strikingly, disrupting the miR-200 sites of the endogenous Zeb1 locus causes a similar phenotype. Reexpressing members of the miR-200 superfamily in vitro reveals that the miR-200c family and not the co-expressed and closely related miR-141 family is responsible for regulation of Zeb1 and EMT. Our results thus show that disrupting the in vivo regulation of Zeb1 by miR-200c is sufficient to drive EMT, thus highlighting the importance of this axis in tumor progression and invasion and its potential as a therapeutic target.

51

Diagnostic potential of microRNA expression profiles in serum and urine of breast and gynecological cancer patients

A. Ritter, M. Jäger, M. Hirschfeld, K. Berner, J. Asberger, D. Weiss, C. Nöthling, G. Gitsch, T. Erbes

Universitätsfrauenklinik Freiburg, Molekulare Onkologie, Freiburg, Deutschland

Purpose: MicroRNAs have been identified as crucial regulators of carcinogenic pathways. Currently, the potential of circulating microRNAs as indicators for various diseases is widely investigated. This in vivo study focused on the putative biomarker properties of microRNAs in the diagnostics of breast cancer (BC), endometrial cancer (EC) and ovarian cancer (OC) to evaluate their potential as minimally invasive biomarkers.

Material and methods: MiRNA microarray chip analysis was performed on serum and urine specimen of BC, EC and OC patients prior to treatment. Therefore, pooled patient samples of both serum and urine were applied for each cancer entity. The breast cancer pool was further stratified according to molecular subtype. MiRNA expression was statistically tested against a control group applying Welch`s two-tailed t-test.

Results: Welch`s two-tailed t-test identified 39 aberrantly expressed miRNAs in serum and 48 miRNAs in urine of the investigated cancer patients. In specific, 14 miRNAs in serum could distinguish between "cancer" and control, 16 miRNAs could indicate BC, 8 miRNAs EC and OC, 17 miRNAs EC and 8 miRNAs OC. Likewise, 10 miRNAs were detected in urine with indicatory potential for "cancer", 7 miRNAs for BC, 11 for EC and OC, 24 for EC and 10 for OC. Some overlapping of miRNAs within the investigated groups has been observed, limiting the number of possible biomarker candidates.

Conclusion: Our results underline the great potential of miRNAs as novel tools in the diagnostic of gynecological malignancies. Due to some overlapping of miRNAs in the performed microarray chip analysis, it might be necessary to identify a unique miRNA signature for each tumor entity in order to increase specificity of potential screening tests for each cancer, respectively. Such signature (or score) would be of immense value and facilitate screening for BC, EC and OC.

52

IL32: a treament to convert tumors from cold to hot

T. Gruber, H. Sadozai, M. Kremenovic, D. Von Werdt, B. Bäriswyl, R. Nives, M. Schenk

Institut für Pathologie, Universität Bern, Schweiz

Immune checkpoint blockade (ICB) using antibodies to the inhibitory molecules CTLA-4 and PD-1, have demonstrated clinical benefit for a number of patients with different types of tumors. Nevertheless, a majority of patients do not achieve long-lasting tumor control using ICB. Recently, several reports have highlighted the crucial role of cross-presenting dendritic cells (DC) in both anti-tumor immunity and response to immunotherapies. IL-32 is a recently described inflammatory cytokine, which has been shown to induce potent cross-presenting DC from human monocytes. Here, we investigated the potential of IL-32 as a tumor immunotherapy in established murine cancer models. IL-32 induced similar transcriptional activities in both human monocytes and murine bone marrow DC. Furthermore, IL-32 matured DC in both humans and mice exhibited enhanced antigen cross-priming in vitro. Subsequently, we observed that intratumoral injections of IL-32 in mice bearing B16F10, MC38 or 4T1 tumors resulted in robust immune cell activation and recruitment to the tumor and significantly delayed tumor growth. This effect was abolished in $BATF3^{-/-}$ mice, which lack putative cross-presenting $CD8a^{+}$ and $CD103^{+}$ DC, and in mice which were depleted of $CD8^{+}$ T cells using monoclonal antibodies. We subsequently performed proteomic assessment of IL32 treated tumors and found that they contained higher concentrations of key immune cell recruiting chemokines such as CXCL-9, -10, and -11, providing mechanistic insight to the hitherto unknown role of IL-32 in the murine immune system. A combination treatment of IL-32 with anti-PD-1 in mice with B16 tumors demonstrated synergistic effects on immune cell infiltration and tumor control. Thus, IL-32 exhibits remarkable potential as a tumor immunotherapy, in particular for patients with low intra-tumor immune cell infiltration who currently fail to respond to ICB.

53

Exploring the molecular mechanisms of UVB-induced cell death in human keratinocytes

G. S. Grossi, H. D. B. Beer

University Hospital Zürich, Clinic for Dermatology, Zürich

Exposure to ultraviolet (UV) light is the most important risk factor for the development of non-melanoma skin cancer (NMSC), the most prevalent type of cancer in Caucasians, with increasing incidence worldwide. Basal cell carcinoma and squamous cell carcinoma account for the majority of NMSC cases and arise from epidermal keratinocytes. In particular, UVB radiation represents a carcinogen, as it is able to induce direct DNA damage, which, if not correctly repaired, can lead to mutations in keratinocytes and subsequently to skin cancer in vivo. On the other hand, UVB-induced apoptosis of the damaged keratinocytes antagonizes the malignant transformation and therefore represents a protective pathway. However, the molecular mechanisms underlying this process are not fully elucidated.

We have previously shown that caspase-1 is required for UVB-induced apoptosis of human primary keratinocytes (HPKs) and aimed to further characterize its role in this process. We established a system based on stable genetic modification of HPKs and inducible overexpression of caspase-1, and employed a proteomics approach termed TAILS for identification of endogenous caspase-1 substrates potentially involved in UVB-induced cell death.

We identified a specific cleavage of the major vault protein (MVP) by caspase-1 at the aspartate 441. MVP is part of huge cytoplasmic ribonucleoprotein particles termed vaults, whose functions are poorly understood. Cleavage of MVP was never described before and, interestingly, we found that this protein is also cleaved by the apoptotic caspase-9. Cleavage of endogenous MVP occurs in HPKs in response to UVB-induced apoptosis and is dependent on expression of caspase-1 and -9. Furthermore, MVP is involved in cell death resistance of HPKs and is degraded during cell death. Overexpression of a cleavage resistant variant of MVP protects the cells against UVB-induced cell death, suggesting an important role of this cleavage event in this pathway.

Our results provide better insights about the role of caspase-1 and -9 in UVB-induced apoptosis and identify MVP cleavage as a novel molecular feature occurring as the result of caspase activation during this cell death process. Further studies will reveal whether MVP has a role in NMSC development.

54

Tracing clonal dynamics reveals that 2D and 3D patient-derived cell models capture tumor heterogeneity of clear cell renal cell carcinoma

H. A. Bolck (1), C. Corro (1), A. Kahraman (1), J. Kuipers (2), N. Toussaint (3), P. H. Schraml (1), H. Moch (1)

(1) Department of Pathology, University Hospital Zurich, (2) Department of Biosystems Science and Engineering, ETH Zurich Basel, (3) NEXUS Personalized Health Technologies, ETH Zürich, Zürich

Recent advances have led to an unprecedented view of the considerable diversity of individual genomes and their evolution among patients with renal cell carcinoma (RCC). Patient-derived cell (PDC) models capable of representing RCC heterogeneity in vitro can serve as valuable tools to gain insights into clonal architecture and how it may be exploited for the management of the disease. Here, we report the generation of a representative biorepository of well-characterized living renal cancer cells models and demonstrate their histopathological and molecular concordance with the corresponding patient tumors. DNA copy number aberrations as well as sequence alterations were consistent between original tumors and PDC cultures and largely retained during serial passaging. Models generated from six different ccRCCs broadly reflected inter-patient genetic diversity. In-depth genetic profiling and clonality analysis of the most simplistic PDC in vitro model, the monolayer cell culture, provided insights into clonal composition and temporal dynamics of ccRCC in vitro culturing and suggested that PDC cultures capture intra-tumor heterogeneity.

The generation of three-dimensional PDC models complemented the diversity of renewable in vitro resources. We show that organoid cultures and microtissues derived from ccRCC patient cells recapitulate histological and genetic features of the original tumors. They could be applied to proof-of-principle drug sensitivity studies with results comparable to the corresponding monolayer cell cultures. We determined that, similar to monolayer PDC cultures, tumor organoids contained multiple subclonal populations reminiscent of the clonal architecture of the corresponding ccRCCs.

Taken together, this study demonstrates that patient-derived cell models mirror inter- and intra-tumor heterogeneity of ccRCCs in vitro. We delineate dynamics of tumor subclones during ccRCC in vitro culturing and suggest that PDC models can be utilized to advance our understanding of the trajectories that generate genetic diversity and their consequences for cancer phenotypes and drug responses on the individual level.

55

NK cells sustain breast cancer dormancy in the liver

A. L. Correia (1), J. C. Guimaraes (2), D. De Silva (1), R. Okamoto (1), M. Bentires-Alj (1)

(1) Department of Biomedicine, University of Basel / University Hospital Basel, Basel, (2) Computational and Systems Biology, Biozentrum / University of Basel, Basel

Failure of the conventional therapies to eradicate dormant tumor cells and prevent tumor recurrence is clearly one of the main barriers to effective cancer treatment. The increasing recognition that dormant disseminated tumor cells (DTCs) depend on the microenvironment within foreign tissues suggests that targeting the latter might be a powerful strategy for therapeutic intervention in metastatic cancer. Deriving and implementing such microenvironmental-targeted therapies in the clinic urges a comprehensive understanding of the cellular and molecular make-up of DTC microenvironments in a tissue-specific manner, which is currently lacking. Here we reveal the liver determinants of metastatic dormancy in a spontaneous metastasis model of breast cancer. We developed a DTC live tracker that distinguishes quiescent from dividing cells and showed the liver is a preferential shelter for quiescent cells, conceding metastatic expansion only in few regional sub-niches. We then isolated hepatic niches corresponding to distinct breast cancer progression stages (dormant disease and manifest metastasis), and reconstructed their cellular composition by next generation sequencing, imaging and immunoprofiling. We identified natural killer (NK) cells as prime effectors of sustained breast DTC quiescence. Depletion of NK cells from early dissemination phase resulted in increased metastatic burden at end stages of cancer progression, whereas boosting this suppressive population prevented hepatic metastatic outgrowths. These findings uncover NK cells as a key unit of the liver dormant niche and show the efficacy of normalizing the microenvironment within distant tissues to prevent metastatic relapse.

56

LATS1 but not LATS2 represses therapy-induced autophagy by a kinase-independent scaffold function

F. Tang (1), R. Gao (1), R. K. R. Kalathur (1), S. Piscuoglio (2), C. K. Y. Ng (2), E. Dazert (3), S. K. Hindupur (3), S. Song (4), A. Hergovich (5), P. Matthias (4), D. S. Lim (6), L. M. Terracciano (2), M. H. Heim (1), M. N. Hall (3), G. Christofori (1)

(1) Department of Biomedicine University of Basel, Switzerland, (2) Institute of Pathology, Basel University Hospital, Basel, Switzerland, (3) Biocenter, University of Basel, Basel, (4) Friedrich Miescher Institute for Biomedical Research, Basel, (5) UCL Cancer Institute, University College London, London, WC1E 6BT, UK, (6) Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea

Autophagy perturbation represents an emerging therapeutic strategy in cancer. While LATS1 and LATS2 kinases, core components of the mammalian Hippo pathway, have been shown to exert tumor suppressive activities, here we report a pro-survival role of LATS1 but not LATS2 in hepatocellular carcinoma (HCC) cells. Specifically, LATS1 restricts sorafenib-induced lethal autophagy in HCC cells, the standard of care for advanced HCC patients. Notably, autophagy regulation by LATS1 is independent of its kinase activity. Instead, LATS1 stabilizes the autophagy core-machinery component Beclin-1 by promoting K27-linked ubiquitination at lysine 32 and 263, thereby promoting inactive dimer formation of Beclin-1. Our study highlights a functional diversity between LATS1 and LATS2 and uncovers a scaffolding role of LATS1 in mediating a cross-talk between the Hippo signaling pathway and therapy-induced autophagy in HCC.

57

The IL-8/CXCR1 axis is associated with cancer stem-like properties and correlates with clinico-pathological features in renal cancer patients

C. Corro (1), M. E. Healy (1), B. Bodenmiller (2), Z. Li (3), P. Schraml (1), A. Weber (1), I. J. Frew (4), M. Rechsteiner (1), H. Moch (1)

(1) University Hospital Zurich, (2) University of Zurich, (3) Harvard Medical School, (4) University of Freiburg

Recent studies suggests that ccRCC possess a rare population of cancer stem cells (CSCs) that might contribute to tumor heterogeneity, metastasis and therapeutic resistance. Nevertheless, their relevance for renal cancer is still unclear. In this study, we successfully isolate CSCs from established human ccRCC cell lines and from cell lines freshly derived from primary ccRCC tumors. CSCs displayed high expression of the chemokine IL-8 and its receptor CXCR1. While recombinant IL-8 significantly increased CSC number and properties in vitro, CXCR1 inhibition using anti-CXCR1ab or repertaxin significantly reduced these features. After injection into immune deficient mice, CSCs formed primary tumors that metastasized to the lung and liver. All xenograft tumors in mice expressed high IL-8 and CXCR1 levels. Further, IL-8/CXCR1 expression significantly correlated with decreased overall survival in ccRCC patients. These results suggest that the IL-8/CXCR1 phenotype is associated with CSC-like properties in renal cancer.

58

Deregulated Interferon-Stimulated Gene 15 (ISG15) leads to DNA replication stress and genomic instability

M. C. Raso, F. Schmid, N. Djoric, L. Penengo

Institute of Molecular Cancer Research, University Zurich, Zurich

The ubiquitin-like modifier ISG15 is an important player of the innate immunity, robustly induced by viral and bacterial infection and highly expressed during inflammation. Importantly, ISG15 is also induced by genotoxic stress and is frequently overexpressed in different types of tumors such as breast, colorectal, renal cancer and melanoma, oral squamous cell carcinoma, bladder and endometrial malignancies. However, despite the clear correlation between ISG15 expression and carcinogenesis, the underlying molecular mechanisms are still elusive.

We uncovered an unprecedented role of ISG15 as fine modulator of DNA replication and genome stability. While normal expression of ISG15 is required to promote proper genome duplication, high levels of ISG15 – as those frequently observed in cancer or upon pathogen infection – promote an abnormal increase of DNA replication speed, resulting in massive DNA double strand breaks and accumulation of chromosomal aberrations. Moreover, cells bearing high ISG15 levels display unrestrained DNA replication and increased chromosomal breakages when challenged with mild doses of replication stress inducers (i.e. camptothecin, cisplatin), suggesting ISG15 as critical modulator of chemotherapeutic response. While investigating the molecular mechanism underlying this regulation, we surprisingly found that ISG15 interacts with key modulators of replication fork remodeling and restart, two processes typically activated in response to genotoxic stress to ensure replisome stability and to prevent forks from collapsing.

Our findings have significant clinical implications, suggesting that pathogen infections or conditions of chronic inflammation may expose to genotoxic stress during DNA replication, with the intrinsic risk of accumulating chromosomal aberrations, predisposing to cancer development. At the same time, these findings open the perspective of exploiting ISG15 and its key functional interactors at replication forks, as sensitizing factors for chemotherapeutic treatments.

59

Novel factors induced apoptosis in human cancer cells; development of new strategies for treatment of cancer

F. Fares, F. Azzam, R. Bar-Shalom, L. Sharvit, B. Fares

Department of Human Biology, University of Haifa, Haifa, Israel

The main goal of the present study is to elucidate the beneficial effects of natural extracts from medicinal mushrooms and plants, indole derivatives and a novel Benzene-Poly-Carboxylic Acid Complex (BP-C1) on growth of human cancer cells. The efficacy of the extracts and compounds on cell growth were tested on human cancer cells originated from; pancreatic, colorectal, breast, prostate, ovary and head and neck cancer in vitro. Moreover, the effect of the componds and extracts on tumor formation were tested in animal models.
The results indicated that all factors are safe and inhibit proliferation and DNA synthesis of the cancer cells in vitro. Moreover, these factors induced cell cycle arrest and apoptosis. Gene expression analysis indicated that the induction of apoptosis was p53-independent and it was through caspase activation and the mitochondrial pathway by releasing cytochrom C and activation of capase 9 followed by activation of caspase 3 and PARP. In vivo studies using xenograft models indicated that treatment of animals with these facors three times a week for five weeks, caused a significant deceleration in the volumes and weights of tumors. This effect was found to be mediated by inducing apoptosis as it was detected by morphological and staining studies. Moreover, pre-treatment of animals with the pre-apoptotic facor, indole derivative, for five weeks before transplanting the cells, significantly reduced tumor development as compared to controls. Tumors were developed in ~80% of controls and ~20% of treated animals. The tumors developed in treated animals were significantly ($p<0.01$) smaller than that developed in controls. These results indicated that natural extracts have no effect on animal weight and liver or kidney functions. The findings of the present study indicated that these agents are not toxic and may prevent tumor development. Thus, it appears that natural derivatives and a novel, BP-C1 Complex, induced apoptosis in human cancer cells and it may offer an effective and non-toxic anti-tumorigenic compounds in humans.

60

Osteosarcoma cells induce fibroblasts differentiation in vitro through extracellular vesicle associated TGF-β

A. M. Mazumdar

Department of Orthopaedics, Balgrist University Hospital/Institute for Biomechanics, ETH Zurich, Zurich

Introduction: Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents with a high propensity for pulmonary metastases, the major cause of death in patients. Hence, understanding the metastatic process is critical for combating patient mortality. It is known that tumor metastasis does not solely depend on the tumour cells but also on the cells in their surroundings. Cancer associated fibroblasts (CAFs) constitute a majority of non-tumour cells in both the primary and metastatic microenvironment. Additionally, they are shown to support tumour cells' survival by secreting pro-tumorigenic growth factors, cytokines and extracellular matrix proteins. It has been demonstrated in several tumor models that tumor-derived extracellular vesicles (30-150nm) (EVs) mediate metastasis by educating distant sites towards a supportive metastatic microenvironment. Furthermore, EVs have also been shown to induce resting fibroblast differentiation into activated myofibroblasts/CAFs with increased α smooth muscle actin (αSMA) expression. Here, we investigate if EVs derived from a panel of human OS cell lines with differential metastatic potential are capable of inducing the differentiation of resting lung fibroblasts into αSMA positive CAFs.

Methods: EVs were purified from cell culture supernatants of 143-B, SAOS, HU09 and M132 OS cells using standard differential ultracentrifugation. 143-B-derived EVs were also isolated from FiberCell's hollow-fiber bioreactor supernatants and further purified with a 30% sucrose cushion during differential ultracentrifugation. Human lung fibroblasts cell lines MRC-5 and Wi38 were treated with PKH67-labelled EVs and EV internalization was assessed by flow cytometry. αSMA RNA and protein expression was evaluated using RT-qPCR, Western Blot and immunocytochemistry. The presence of LAP-TGF-β on the surface of EVs was determined through flow cytometry. Phosphorylation of SMAD2 in lung fibroblasts was determined by Western Blot analysis.

Results: MRC-5 and Wi38 fibroblasts efficiently internalized EVs in a time- and concentration-dependent manner. Exposure to highly

aggressive 143-B-derived EVs caused differentiation of both fibroblast cell lines into an activated phenotype with increased αSMA expression. On the contrary, exposure to other OS derived cell lines did not activate the fibroblasts. 143-B EVs also increased fibronectin production in both fibroblast cell lines.
We postulated that EV-associated TGF-β was responsible for fibroblast activation. Indeed, we could detect membrane associated LAP-TGF-β on the surface of 143-B-derived EVs membranes. Moreover, we could demonstrate SMAD2 phosphorylation in both fibroblast cells after 60 and 120 minutes of 143-B-derived EV exposure. Finally, pretreatment of fibroblasts with TGFBR1 inhibitor SB-431542 prevented 143-B-derived EVs from activating both fibroblast cell lines.

Conclusion: Our in vitro experiments showed that TGF-β associated with 143-B-derived EVs activates lung fibroblasts. These results indicate a possible role of OS-derived EVs in facilitating pre-metastatic niche education through the activation of niche-resident fibroblasts into CAFs. We have planned future in vivo studies which could further broaden our understanding of how EVs contribute to the pre-metastatic niche foundation and hence open new avenues for OS management.

61

Effect of IgH/c-myc translocation, de novo introduced using CRISPR/CAS9 RNPs, on phenotype of primary EBV-infected B cells

S. Sidorov, S. Boulnom, M. Bernasconi, D. Nadal, C. Berger, S. Bürgler

Experimental Infectious Diseases and Cancer Research, University Children's Hospital of Zürich, Zürich, Switzerland

Burkitt lymphoma (BL) is an aggressive B cell cancer which is characterized by the IgH/c-myc translocation. However, co-factors, such as additional mutations and infections are also required for BL development. Epstein-Barr Virus (EBV) is found in biopsies of almost all cases of endemic BL, suggesting its important role in BL pathology. However, exact contribution of EBV to BL development, as well as the interplay between growth programs induced by EBV and c-myc, is still not well understood.

In order to clarify the effect of IgH/c-myc translocation on EBV-infected cells during early BL development, we devised a novel method to introduce and select for the translocations of interest in model of EBV+ pre-BL cells. As a model of EBV+ pre-BL cells we used primary B cells infected and transformed in vitro with EBV to generate lymphoblastoid cell lines (LCLs). In order to introduce the IgH/c-myc translocation, we generated dsDNA breaks near c-myc and IgH genes in LCLs using CAS9 Ribonucleoprotein (RNPs), and promoted the translocation by adding a GFP-encoding ssDNA template with homology regions to both IgH and c-myc.

We were able to show that ssDNA repair template can deliver and integrate the sequence of interest into the translocation junction leading to GFP expression. Then we assessed changes in phenotype of EBV-infected cells induced by the IgH/c-myc translocation. We could not observe any significant alterations induced by translocation alone. Therefore, we are currently investigating whether siRNA suppression of EBV growth program in EBV-infected cells containing translocation would induce additional changes in EBV-infected cells and generate more BL-like phenotype.

In conclusion, we have established a robust protocol to efficiently induce specific chromosomal translocations by CRISPR/CAS9 technology, which can be easily adapted to model other common malignant translocations. Furthermore, we generated a novel model of IgH/c-myc translocation in pre-BL cells, which has a number of advantages over existing models, relying on stable transfection with c-myc/IgH-containing plasmids: a translocation between endogenous c-myc and IgH gene is induced, pre-existing epigenetics landscape is maintained and all relevant enhancers/regulators are in place and at physiological distance from c-myc gene.
Finally, observation that IgH/c-myc translocation alone is not sufficient to get BL from EBV-infected cells indicates that additional, yet unidentified factors are involved in disease development, suggesting further topics for investigation.

62

A clear cell renal cancer metastasis model identifies novel mediators of tumor aggressiveness and predictors of patient survival

F. Geist (1), T. Dolt (1), T. Höfner (5), C. Klein (2), V. Vogel (2), A. Stenzinger (3), T. Holland-Letz (4), O. Kossi (2), W. Weichert (6), P. Schirmacher (3), S. Pahernik (7), M. Hohenfellner (5), A. Trumpp (1), M. R. Sprick (2)

(1) Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany, (2) Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM), 69120 Heidelberg, Germany, (3) Department of Pathology, University Heidelberg, 69120 Heidelberg, Germany, (4) Department of Biostatistics, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany, (5) Department of Urology, University Heidelberg, 69120 Heidelberg, Germany, (6) Institute of Pathology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Strasse 22, 81675, Munich, Germany, (7) Department of Urology, University Hospital Nuremberg, Nuremberg, Germany

Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cell carcinoma with a 5-year survival rate of only 10.5%, and chemotherapy and radiotherapy regimen have shown limited efficacy. On the molecular level, significant inter- and intra-tumor heterogeneity hamper identification of drug targets, biomarkers and unraveling of disease mechanisms.

We developed a novel patient-derived model system of ccRCC that recapitulates the heterogeneity of the originating cancer enabling us to study ccRCC on a functional level. In five rounds and in three biological replicates of an in vivo selection, we transplanted the metastases of orthotopically transplanted tumor cells into the renal capsules of NOD scid gamma (NSG) mice. The tumor was enriched for cells with higher growth and metastatic potential compared to the initial heterogeneous population. Comparative gene-expression analysis revealed candidate genes associated with enhanced malignant growth and metastasis. Absolute shrinkage and selection operator (LASSO) regression identified a gene signature that can robustly predict cancer specific patient survival. The prognostic power of our signature was additionally verified in independent patient cohorts suggesting that this approach leverages efficient stratification of patients into distinctive risk groups.

One of the hallmark genes in this signature is known to alter cellular signaling properties. Therefore, we hypothesized that this gene contributes to tumor growth and metastasis and thus to aggressiveness of ccRCC. In fact, in knockdown and overexpression xenografts experiments we could confirm an essential role for tumor aggressiveness in vivo suggesting that the gene and associated downstream signaling pathways are attractive targets for treatment of clear cell renal cancer.

63

Identification and targeting of the MAP4K4 effector mechanisms that control brain tissue infiltration in Medulloblastoma

J. Migliavacca (1), C. Capdeville (1), M. T. Schönholzer (1), M. Grotzer (2), M. Baumgartner (1)

(1) Paediatric Neuro-Oncology group, Department of Oncology, Children's Research Center, University Children's Hospital Zürich, August-Forel Strasse 1, CH-8008 Zürich, Switzerland, (2) Department of Oncology, University Children's Hospital Zürich, Steinwiesstrasse 75, CH-8032 Zürich, Switzerland

Migration and invasion of Medulloblastoma (MB) tumor cells causes metastatic disease that is associated with poor prognosis and high morbidity. Blocking tumor cell migration and local infiltration could restrict tumor progression and metastasis. However, molecular mechanisms leading to aberrant migration and tissue invasion in MB are poorly understood and no targeted anti-metastatic therapies exist till date.
We have identified the Ser/Thr protein kinase MAP4K4, which we found up-regulated in primary MB samples, as a mediator of MB cell migration and invasion downstream of receptor tyrosine kinases (RTKs). We hypothesized that the transmission pro-invasive functions of RTKs by MAP4K4 is the consequence of its capability to modulate the cortical F-actin cytoskeleton and membrane dynamics, which promotes invasive cell protrusions at the leading edge of MB cells.

Our objectives are to determine how MAP4K4 controls invasive protrusions and tissue infiltration and to design means to target it. To identify relevant effectors of MAP4K4 in MB, we use pull-down approaches followed by mass-spectrometry in unstimulated and RTK-stimulated cells. Strong interactors are isolated using FLAG-tag pull-down, weak or very transient interactors are identified using proximity-dependent biotinylation (BioID). Towards that, we have

established MB cells lines expressing FLAG-tagged MAP4K4 or N- and C-terminal fusions of MAP4K4 with BioID2. We have identified a number of putative MAP4K4 effectors and demonstrate that endogenous proteins can be biotinylated in MB cells.
We will discuss the specificity of this approach and the potential significance of proteins identified in the context of invasion control.

64

Characterisation of cancer cell response to CXCL8

E. A. Alassaf, A. M. Mueller

Pharmacy, University of East Anglia, Norwich

Introduction: Chemokines such as CXCL8 (IL-8), are defined by their ability to induce directed cell migration. CXCL8 and its cognate receptors (CXCR1 and CXCR2) mediate the initiation and development of various cancers [1]. The aim of this study is to analyse the effect of CXCL8 on MCF-7 (breast), MDA-MB231 (breast), PC3 (prostate) and THP-1 (monocyte leukaemia) cell lines.

Methodology: THP-1 (25x104/mL) were loaded into a microchemotaxis chamber and stimulated with 5 nM CXCL8 for 4 hours. MDA-MB231 cells (1x106/mL) were stimulated with 10 nM CXCL8 overnight in Boyden chamber; migratory cells were stained with Calcein. MCF-7 monolayers were scratched and incubated with 10 nM CXCL8. Wound closure was quantified after 24 hours. MCF-7 and THP-1 cells in calcium flux dose-response assay were loaded with Fura-2AM and calcium release was measured upon injection of CXCL8. MDA-MB231 cells were grown sparsely in the individual cell tracking assay and imaged for 40 hours. PC3 cells (5x104) migration towards CXCL8 was quantified using individual cell tracking assay imaged for 10 hours and Oris migration assay. Detection of receptors expression was performed with anti-CXCR1/2 in immunofluorescence assay.

Results: CXCL8 significantly stimulate MCF-7 migration in wound healing assays ($P \leq 0.05$, n=4) as well as stimulating calcium release (EC50= 364 nM). THP-1 cells migrate towards CXCL8 and release calcium (EC50= 271 nM). CXCL8 induce migration in MDA-MB231 cells in individual cell tracking and Boyden chamber ($P=0.04$, $P<0.0001$, n=3, 8 respectively). PC3 can migrate towards CXCL8 ($P \leq 0.05$, n=3). MDA-MB231 and PC3 express CXCR1/2, THP-1 express CXCR2 and MCF-7 showed heterogeneity of expression.

1. Q. Liu, A. Li, Y. Tian, J. D. Wu, Y. Liu, T. Li, Y. Chen, X. Han, and K. Wu, "The CXCL8-CXCR1/2 pathways in cancer," Cytokine Growth Factor Rev., vol. 31, pp. 61–71, 2016.

65

Microenvironmental T-helper cells increase APOBEC off-target mutagenesis in BCP-ALL cells

S. Traxel, L. Schadt, J. Lehmann, C. Berger, D. Nadal, S. Bürgler

Experimental Infectious Diseases and Cancer Research, Children's Research Center, University Children's Hospital Zurich, Switzerland

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common childhood malignancy and arises from expansion of precursor B-cells in the bone marrow. Although current treatment has greatly improved the survival of BCP-ALL patients, relapses still present a major challenge in the treatment of BCP-ALL due to resistance to treatment. Relapses are associated with mutations in various genes. In BCP-ALL the mutational signature is partially caused by cytidine deaminases. In mature B-cells, activation-induced cytidine deaminase (AID) is an important cytidine deaminase which causes beneficial mutations in immunoglobulin genes and which gets upregulated upon T-helper (Th) cell stimulation. In precursor B-cells, AID is usually not expressed but is associated with worse prognosis in BCP-ALL. Interestingly, we previously showed that autologous Th-cells interact with BCP-ALL cells like they do with mature B-cells by inducing activation and proliferation. Therefore, we hypothesize that Th-cell-derived stimuli induce AID expression in BCP-ALL cells and thereby lead to increased off-target mutagenesis. Using qRT-PCR and cytometric bead array, we determined the cytokine profile of Th-cells derived from leukemic bone marrow. We found that IFNγ, but also the known AID-inducers TGFß and IL-13 were expressed. In vitro TGFß, IL-13 and CD40L - mimicking cell-cell contact - increased the expression of AICD Aboth in primary BCP-ALL cells and cell lines as shown by qRT-PCR. To assess AID activity in BCP-ALL cells, we established a reporter system where cytidine deaminase activity leads to GFP expression. Stimulation of a BCP-ALL cell line with TGFß, IL-13 and CD40L led to increased GFP expression, indicating an increased mutagenesis. We therefore suggest that bone marrow Th-cells interact with BCP-ALL cells, induce mutations and thereby contribute to the aggressiveness of BCP-ALL. Our identification of Th-cell-derived factors as inducers of AID expression and activity might therefore open up new targets for treatment to avoid relapse-associated mutations.

66

The Growth Differentiation Factor 15 (GDF15) is a Novel Target Gene for Oncogenic Human Papillomaviruses

J. Mändl, K. Hoppe-Seyler, F. Hoppe-Seyler

Molecular Therapy of Virus-Associated Cancers, German Cancer Research Center (DKFZ), Heidelberg, Germany

Worldwide more than 500,000 women develop cervical cancer each year. Two oncoproteins of human papillomaviruses (HPVs), E6 and E7, are the driving forces for carcinogenesis by inducing the degradation of the p53 and the pRb tumor-suppressor proteins, respectively. In search of further host cell factors targeted by oncogenic HPVs, the growth differentiation factor 15 (GDF15) mRNA showed the highest upregulation upon silencing of endogenous E6/E7 expression in a transcriptome array of the HPV18-positive cancer cell line HeLa. This implies that oncogenic HPVs may repress GDF15 expression.

Like other members of the transforming growth factor beta superfamily, GDF15 functions as a cytokine and is induced after various forms of stress, such as endoplasmatic reticulum (ER) stress or treatment with non-steroidal anti-inflammatory drugs (NSAIDs). While its downstream targets are still diversely discussed, GDF15 is reported to mediate pro-apoptotic processes. Therefore, HPV-positive cancer cells may benefit from a downregulation of GDF15 expression in order to promote cellular stress resistance.

In line with the results of the transcriptome array, the decrease of GDF15 mRNA and protein levels through the viral oncogenes was verified by independent methods, such as qRT-PCR and immunoblot analyses in both HPV16- and HPV18-positive cancer cell lines. Notably, GDF15 expression is no longer activated upon E6/E7 repression in a HeLa-derived cell line in which the TP53 gene is efficiently silenced by a stably expressed shRNA. Further, we show that p53 is involved in the increase of GDF15 levels after NSAID or ER stressor treatment in both HPV-positive and negative cancer cells. Ongoing experiments indicate that the knockdown of GDF15 expression in HeLa by RNA interference reduces cleavage of PARP after the addition of thapsigargin, which point to an inhibition of apoptotic processes triggered by the ER stressor. Live cell imaging experiments suggest that the phenotypic effects of GDF15 are stress-dependent, since the downregulation of GDF15 in the absence of stress inducers does not affect the proliferation of HPV-positive cells. Collectively, these data indicate that the HPV E6 oncoprotein can elevate the stress resistance of cervical cancer cells by interfering with the p53-mediated stimulation of the pro-apoptotic GDF15 gene. Future experiments will address the significance of this newly discovered regulatory principle for the clinically relevant question whether the inhibition of GDF15 expression by E6 may also increase the resistance of HPV-positive cancer cells towards chemo- and radiotherapy.

67

Role of mutsβ protein in maintenance of genomic stability

E. Isik, P. Janscak

Institute of Molecular Cancer Research, University of Zurich, Zurich

Faithful segregation of completely and accurately replicated DNA to daughter cells is essential for the maintenance of genome stability. Nevertheless, endogenous or exogenous obstacles stall or slow the progression of replication forks, a condition known as replication stress. The entry into mitosis with persistent replication intermediates impairs chromosome segregation and leads to genomic instability, a hallmark of cancerous cells. Recent studies have shown that collisions between DNA transcription and replication machineries in a head-on orientation constitute a major endogenous source of replication stress. Replication fork stalling results from the formation of RNA:DNA hybrids between the nascent transcript and the template DNA strand giving rise to a structure termed R-loop. The molecular mechanisms and major players in the resolution of transcription-replication collisions and restart of DNA replication at R-loop-stalled replication forks remain elusive. MutSβ is a heterodimeric protein of MSH2 and MSH3 subunits that acts as a mismatch-binding factor during post-replicative mismatch repair (MMR). In this project, we aim to investigate the role of MutSβ in the resolution of transcription-replication collisions. Our preliminary data revealed that MSH3-depleted cells accumulate chromosomal breaks upon treatment of cells with camptothecin (CPT), which is known to promote interference between transcription and replication by inducing R-loop formation. Interestingly, these breaks are dependent on the MUS81 endonuclease that is required for restart of R-loop-stalled forks. Using DNA fiber spreading assay, we have found that PARP inhibition could not rescue CPT-induced replication fork slowing in MSH3-depleted cells. Thus, our ongoing studies suggest a role for MutSβ in replication fork restart at R-loops, downstream of MUS81. We would like to extend our studies to better understand the role of MutSβ in the replication restart process at co-transcriptional R-loops. Full mechanistic understanding of these processes can reveal novel therapeutic approaches to improve the efficiency of currently used chemotherapeutic treatments of cancer.

68

SPIDA - Surface protein internalization and degradation assay

F. Kast, J. C. Stüber, F. Andres, A. Plückthun

Department of Biochemistry, Faculty of Science, University of Zurich

Due to being signaling hubs, key regulators of fundamental cellular processes and thus upon dysregulation often at the root of human diseases, cell surface proteins are the target of numerous molecules, which are approved, in clinical trials or in preclinical development. A well-established negative feedback loop of natural ligands is the down-regulation of their cognate receptors. However, active internalization and possible degradation by drug candidates is only recently gaining more attraction. Today, affinity reagents like antibodies and DARPins are selected in high throughput. Therefore, characterization of selection output necessitates methods achieving similar levels to prevent the analysis from becoming a major bottleneck in the discovery pipeline.

Surface Protein Internalization and Degradation Assay (SPIDA) is homogenous generic high-throughput assay, allowing to simultaneously and independently quantify internalization and degradation rates of surface proteins on a single-cell level, without requiring labeling of the molecules to be screened. The assay may be performed over at least 24 hours with adherent live cells in a 96-well format. We fuse a HaloTag to the cell surface protein of interest and exploit the differential cell permeability of two fluorescent HaloTag ligands to allow binary distinction of localization in a flow cytometry based protocol. True relative abundances of surface and internal protein are obtained through channel rescaling, and we are further able to calculate total protein.

We set up and validate our assay with two major drug targets of the epidermal growth factor family, EGFR and HER2, examining a selection of well-investigated, but also novel small molecule ligands and protein affinity reagents. Our unique bispecific antibody for example shows pronounced internalization and degradation of HER2. We furthermore characterized bispecific DARPins targeting different epitopes on cMET and here as well show degradation of the receptor as main mode of action. Eventually, we analyzed EpCAM binding DARPins, which were previously equipped with a MMAF warhead and tested as antibody drug conjugates in preclinical studies.

69

Apoptosis-inducing anti-HER2 agents operate through receptor lockdown

J. C. Stüber (1), C. P. Richter (2), M. Schwill (1), I. König (1), Y. Waltenspühl (1), B. Schuler (1), J. Piehler (2), A. Plückthun (1)

(1) Department of Biochemistry, Faculty of Science, University of Zurich, (2) Department of Biology, University of Osnabrück

The overexpression of receptor tyrosine kinases (RTKs) at the cell surface leads to hyperactivation of downstream signaling pathways and drives the growth of various tumors, but also can confer resistance against conventional targeted agents, and thus represents a major challenge for today's clinical therapy. While the RTK HER2 has become a prime target of antibodies, small molecules and ADCs, disease progression and development of cancer drug resistance is still a pressing issue in treatment of HER2-positive cancers.

Previously, we reported biparatopic HER2-binding Designed Ankyrin Repeat Proteins (DARPins), which are able to completely block the growth and induce apoptosis in HER2-dependent cancer models, exhibiting anti-tumor activity superior over the conventional mAbs trastuzumab and pertuzumab.

Here, we present a comprehensive analysis of HER2 organization and downstream signaling, as well as their modulation by different targeted drugs. By employing fluorescence-based methods, we investigated the modulation of receptor dynamics and found a clear correlation between reduced HER2 mobility and the effectiveness of anti-HER2 drugs. We show that "lockdown" of HER2 by biparatopic agents involves the formation of large HER2 complexes, anchored to immobile receptors. These clusters are incompetent to initiate a signaling pathway rewiring known to drive adaptive resistance against mAbs and small molecules, preventing the re-activation of AKT-signaling by RAS-PI3K crosstalk. Our findings underpin that potent and sustained neutralization of RTKs is possible through the formation of geometrically well-defined complexes by biparatopic binding agents, and link differential surface receptor reorganization through the treatments with specific signaling outputs.

70

Serum chemokine profiling reveals candidate biomarkers for recurrence and immune infiltration in ovarian cancer

A. Mlynska, G. Salciuniene, K. Zilionyte, B. Intaite, A. Barakauskiene, V. Pasukoniene

Laboratory of Immunology, National Cancer Institute, Vilnius

Depending on the strategy employed by tumors to avoid immune surveillance, several immune phenotypes emerge: immune desert, immune exclusion, and inflammation, each with its unique immune contexture. The cross-talk between cancer and immune cells is orchestrated by cytokine and chemokine network, which can act both locally and systemically. Molecular analysis of ovarian cancer highlighted the relevance of the immune system in tumor development and response to treatment. Despite frequent recurrence and limited effective chemotherapy treatment options, the selection of reliable prognostic and predictive biomarkers, especially those of immune origin, in ovarian cancer remains limited. We hypothesized that chemokines in ovarian cancer patient serum may reflect the immune tumor microenvironment status and serve as potential biomarkers for personalized management of ovarian cancer.

We used the TCGA dataset to explore the mRNA expression in three distinct immune subtypes. Next, from the 40 stage III-IV ovarian cancer patients cohort, we collected preoperative serum samples and surgically removed tumor tissue. We evaluated the level of immune tumor infiltration by histology staining and flow cytometry. We measured the preoperative level of intratumoral and circulating chemokines to select the combinations allowing to predict the immune-infiltrated and recurrent tumors.

Supervised K-means clustering analysis revealed three clusters of patients from the TCGA dataset with differential gene expression, each characterized by the unique chemokine signature. In patients' cohort, we detected the increased levels of CXCL11, CCL2, CXCL10, and CXCL9 in the preoperative serum of patients with immune-infiltrated versus non-infiltrated tumors. After grouping the patients based on their disease recurrence status, we detected the increased levels of CCL20, CXCL1, CCL3, CCL4 in the preoperative serum of patients with recurrent disease versus non-recurrent disease. We next carried the ROC analysis of multiple chemokines to select best-performing combinations.

Our results suggest that high preoperative levels of circulating CXCL9+CXCL10 chemokine combination in ovarian patients serum can distinguish immune-infiltrated tumors, whereas high preoperative levels of circulating CCL4+CCL20+CXCL1 chemokine combination in can predict the recurrence of the disease.

71

Relative copy number: a new strategy for integrative analysis of copy number aberration in cancer

B. Gao, M. Baudis

Institute of Molecular Life Sciences, University of Zurich, Zurich

Chromosomal copy number aberrations (CNA) are frequently observed in the cancer genome. They can provide valuable supporting information in both cancer research and clinical practice. Currently, CNAs can be directly measured with SNP arrays or imputed from sequencing data. Although the measurement result is straightforward, the interpretation is difficult for both single sample and comparative analysis. This is mainly caused by two reasons: first, the CNA calling outcome is the calibration result of signal strength instead of the true copy numbers. Second, a tumor sample almost always contains a certain amount of normal cells and sub-clones. Ideally, the copy number of a chromosome segment should be an integer, because of the aforementioned reasons, the actual measurement result is always noised and deviates from the true integer value. Thus, it is very important to identify the true copy numbers in order to have proper interpretation of single samples and comparisons in integrative analysis. Unfortunately, this is an extremely challenging task as the key information regarding cell compassion of the sample is lost in the early steps of the sequencing process. A few numbers of statistical or mathematical methods have been developed to tackle this problem, but all with their drawbacks and limitations. In this study, instead of pursuing the true copy numbers, we propose a new strategy that uses relative measurement to calibrate signal strength. In this way, we can make confident interpretation of single samples and perform normalization for integrative analysis without knowing the true copy numbers.

72

Systemic Analysis of Tyrosine Kinase Signaling Reveals a Common Adaptive Response Program in a HER2-positive Breast Cancer

M. Schwill (1), R. Tamaskovic (1), A.S. Gajadhar (2), F. Kast (1), F.M. Forest (2), A. Plückthun (1)

(1) University of Zurich, (2) Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology

Drug-induced compensatory signaling and subsequent rewiring of cancer signaling promote the development of acquired cancer drug resistance, which ultimately leads to escape from targeted therapy. Here we sought to analyze the early adaptive kinase response after distinct treatments against HER2, which induce either temporary G0/1 cell cycle arrest or apoptosis in HER2-overexpressing cancers. We compared treatment with trastuzumab, ARRY-380, their combination and a biparatopic anti-HER2 DARPin and characterized the HER2-driven tyrosine kinase signaling network based on a proteome-wide phosphopeptide quantification by isobaric tagging multiplex (TMT) LC-MS/MS. Next, we analyzed the feedback activation of serine/threonine and tyrosine kinases after treatment using a bait peptide chip array and predicted the corresponding active kinases from the specific substrate phosphorylation profiles. By combined system-wide analysis of both large-scale datasets, we identified a common adaptive kinase response program, which is similar between the mechanistically different anti-HER2 treatments and essentially involves activation of FAK1, PKCδ and EPH family receptors. We found that the treatment-induced patterns in the overall tyrosine phosphorylation are characteristic for the mechanism of a drug; yet, the time-dependent phosphorylation trends of a specific peptide signature are predictive for the treatment-induced cancer cell fate. Furthermore, we identified a counter-regulatory phosphorylation between the AKT and FAK1 signaling pathways upon HER2/HER3 inhibition. Finally, we found a significant additive effect of an anti- HER2 and FAK1 combination treatment in HER2-positive cancers.

Significance: The complex process of development of cancer drug resistance from an initially responsive state to the state of drug-resistance is not understood today. This study sheds light on the early signaling events after the perturbation of the primary oncogene HER2 by various mechanistically different anti-HER2 drugs in a HER2-dependent breast cancer model. In response to the various treatments, we observed significant differences in the intensity of the adaptive kinase activation but our systemic analysis revealed a common pattern of active kinases throughout these different treatments. Thus, the early adaptive cancer drug response follows a defined minimal resistance program in our HER2-dependent model, which is fairly independent of the treatment and depends on the activation of a small number of critical kinases. We identified FAK1 kinase as a key player in this adaptive response program and we could show that different anti- HER2 and FAK1 combination treatments were additive in their effectiveness of inducing cancer cell death. Thus, these drug combinations did overcome the adaptive compensatory kinase signaling and offer a new opportunity for improved treatment of drug resistant HER2-positive breast cancers.

Background: Previously, we developed a new class of biparatopic anti-HER2 binding agents, biparatopic DARPins (e.g., DARPin 6L1G), which blocked productive HER2 receptor homodimer- and heterodimer- interactions and, consequently, induced apoptosis in various HER2-dependent breast cancer models (Tamaskovic et al., 2016; Jost et al., 2013). These biparatopic agents, similar to the trastuzumab plus lapatinib or trastuzumab plus ARRY-380 combination treatment, can overcome the adaptive kinase response signaling and effectively induce apoptosis in HER2-dependent cancer cells, which confirmed the existence of HER2 oncogene addiction and explained that it needs to be overcome by simultaneous blockade of signaling from HER2 and HER3.

73

HIF-1 Drives the Assembly of the Multi-Enzyme Purinosome Complex in Hypoxic Cancer Cells

C. Doigneaux (1), A. M. Pedley (2), I. N. Mistry (1), S. J. Benkovic (2), A. Tavassoli (1)

(1) School of Chemistry, University of Southampton, Southampton, SO17 1BJ, U.K., (2) Department of Chemistry, The Pennsylvania State University, University Park, PA 16802, U.S.A.

The purinosome is a dynamic metabolic complex composed of the six enzymes of the de novo purine biosynthesis. This multi-enzyme complex was previously shown to assemble in response to elevated purine demand. An increase in purine demand is often observed in neoplastic cells; however, the physiological conditions that govern purinosome formation in these cells remain unknown. Here, we report that cells readily form purinosomes when placed in a low oxygen microenvironment (hypoxia). We demonstrate that this process is driven by, and dependent on the activation of hypoxia inducible factor 1 (HIF-1). Analysis of intracellular metabolites shows that purinosome assembly serves to increase the intracellular purine pool in hypoxic cancer cells. Our findings link two common hallmarks of cancer, hypoxia and upregulated glycolysis, to increased biosynthesis of purine metabolites required for cellular division and function. For the first time, we show this connection is achieved via up-regulation of metabolon assembly, which tantalizes the possibility that inhibition of purinosome formation may serve as a potential therapeutic approach for targeting hypoxic cancer cells.

74

Roles of the Epithelial-to-mesenchymal transition states in drug resistance

N. Sugiyama (1), F. Lüönd (1), R. Bill (1), H. Oller (2), P. Pelczar (2), G. Christofori (1)

(1) Department of Biomedicine, University of Basel, 4058 Basel, Switzerland, (2) Centre for transgenic models, University of Basel, 4058 Basel, Switzerland

Tumor recurrence after chemotherapy remains a major clinical challenge. Epithelial-to-mesenchymal transition (EMT) is a transdifferentiation program, which contributes to tumor malignancy by not only enhanced mobility and invasion but also generating intra-tumor heterogeneity and resistance to cancer therapy. Since EMT is a transient process, it has not been fully elucidated what the implications of different EMT states are for drug resistance. Here we established three-dimensional (3D) organoid cultures derived from our genetic mouse models that allow the lineage tracing of breast cancer cells undergoing either "early" or "late" stage of an EMT by switching their color from red to green upon tamoxifen administration. In organoids derived from the early-EMT lineage-tracing mouse model, green tumor cells localized to the organoid-ECM border and formed large, poorly differentiated structures. In the late-EMT lineage tracing system green-color cells were very rare and found exclusively at the invasive front. These results suggest that epithelial-mesenchymal hybrid cells may critically contribute to tumor outgrowth, while a full EMT from bona fide epithelial to bona fide mesenchymal cells may be a rare event, and bona fide mesenchymal cells would play a key role in tumor invasion. We further generated either cyclophosphamide- or paclitaxel-resistance 3D organoids. These organoids were incubated with tamoxifen to induce color switching prior to the drug treatment. In control and cyclophosphamide-resistant "partial" EMT organoids, the number of green-color cells was much smaller than that of red-color cells, whereas majority of the cells were in green in the paclitaxel-resistant organoids. Interestingly, treatment with higher dose of paclitaxel could induce EMT-like phenotypic change of the resistant organoids, suggesting dose-dependent effects of this drug on tumor. In contrast, chclophosphamide treatment induced disassembly of F-actin cytoskeleton. In organoids derived from the late-EMT lineage-tracing mouse model, green-color cells were rarely found and their number was not altered by either cyclophosphamide or paclitaxel treatment. These results suggest that the cells that have undergone "partial" EMT have greater resistant ability to paclitaxel, and paclitaxel could also induce EMT-like phenotypic change in collagen-rich 3D condition. In contrast, EMT may not contribute to the resistance to cyclophosphamide.

75

DNA methyltransferase inhibitors prevent emergence of acute myeloid leukemia chemoresistance by suppressing relapse of leukemia-initiating single-cell lineages

F. C. Caiado (1), D. M. S. Maia-Silva (1), C. J. Jardim (1), T. C. Carvalho (1), C. R. Reforço (1), R. F. Faria (1), B. K. Kolundzija (1), A. E. S. Simões (1), N. S. Schmolka (1), M. Gomes da Silva (2), T. N. Schumacher (3), H. N. Norell (1), B. Silva-Santos (1)

(1) Instituto de Medicina Molecular, Lisbon, (2) Instituto Português de Oncologia, Lisbon, (3) Netherlands Cancer Institute, Amsterdam, Netherlands

Chemotherapy resistant cancer recurrence is a major cause of mortality. In acute myeloid leukemia (AML) chemoresistant relapses result from genetic evolution and/or transcriptional alterations resulting from aberrant DNA methylation. Nevertheless, the underlying tumor cell population dynamics that drive these developments through the relapse process remains largely unknown. We found that combining standard chemotherapeutic regimens with clinically available DNA methyltransferase inhibitors (DNMTi; Decitabine – DAC and Azacitadine - Aza) prevents the emergence of chemoresistant escape variants in AML that recur after therapy. Lineage tracing of barcoded human AML cells revealed that chemotherapy consistently selected for a pre-determined set of single-cell lineages that was targeted by low-dose DNMTi combination, leading to dominance by originally rare and unpredictable lineages that remained chemosensitive after relapse. Genomic analyses indicated a stable genetic composition after all therapies, while the DNMTi combination mechanistically achieved its impact by preventing senescence-associated transcriptional stemness (SAS) programs in the relapses. Furthermore, DNMTi combination depleted human leukemia stem cells and lineages with enhanced leukemia-initiating potential in vivo. Our findings suggest that upfront combination of chemotherapy with DNMTi targets a predetermined set of chemoresistant single-cell lineages with increased leukemia-initiating potential, inheritable at kinship levels beyond genetics, thereby preventing the development of stemness-dependent chemoresistant AML relapses.

76

Endocytosis: Its regulation and functional relevance for the establishment of the pro-invasive phenotype in Medulloblastoma

C. Capdeville (1), L. Russo (1), D. Tripolitsioti (1), J. Migliavacca (1), M. Grotzer (2), M. Baumgartner (1)

(1) University Children's Hospital Zürich, Department of Oncology, Children's Research Center, Zurich, Switzerland, (2) University Children's Hospital Zürich, Department of Oncology, Zurich

Medulloblastoma (MB), the most common malignant brain tumor in childhood, locally infiltrates and forms distant metastases. Despite intensive therapy protocols, one third of the patients succumb to their disease and successful treatment is often associated with long-term sequelae and high morbidity in patients. Approaches that specifically target infiltration and metastatic dissemination are needed to increase efficacy of existing treatments and to de-escalate current protocols.

We found that the serine/threonine protein kinase MAP4K4 mediates growth factor (GF)-induced cell migration and invasion in MB. We demonstrated that the GF HGF promotes endocytic uptake in a MAP4K4-dependent manner in MB cells and that pharmacological and genetic blockade of endocytosis restricts migration. We hypothesized that MAP4K4 controls the endocytic uptake triggered by HGF and that this function of MAP4K4 is an essential mechanism for the cells to migrate and invade.

To identify mechanisms controlling endocytosis in MB cells, we use quantitative imaging and biochemical approaches and adapt them to high-throughput analysis. Using an siRNA approach, we furthermore identify relevant endocytic pathways promoting the invasive phenotype of MB cells. We identified endophilin A subfamily as a critical regulator of HGF-induced migration and currently explore its functional significance in endocytic regulation in general and in the context of cell invasion controlled by MAP4K4.

We expect form these studies relevant insights in the contribution of altered endocytic activity to the establishment of the pro-metastatic phenotype in MB and mechanistic understanding of the underlying processes, which will guide the rational design of novel anti-metastatic therapy approaches in MB.

77

FOXM1 and CKS1 - novel cellular targets of the HPV oncogenes

S. Adrian, K. Hoppe-Seyler, F. Hoppe-Seyler

Molecular Therapy of Virus-Associated Cancers, German Cancer Research Center (DKFZ), Heidelberg, Germany

Cervical cancer is caused by persistent infection with oncogenic human papillomaviruses (HPVs) and remains a major cause of cancer-related death in women worldwide. Tumorigenesis is driven by the viral E6 and E7 oncoproteins which inactivate the tumor suppressor proteins p53 and pRb. Inactivation of p53 and pRb, however, is not sufficient for HPV-induced cell transformation. Thus, the identification of additional cellular factors which are dysregulated by E6/E7 is of critical importance to better understand this process, and could lead to new therapeutic options. FOXM1 (Forkhead Box Transcription Factor M1) is a cellular oncoprotein that contributes to carcinogenesis and therapeutic resistance. It is overexpressed in a majority of human cancers, which is linked to a worsened clinical prognosis. A potential downstream target, which is activated by FOXM1, is the cell cycle regulator and proto-oncogene CKS1B (Cyclin-dependent Kinase Regulatory Subunit 1B).

We detected in transcriptome analyses that both FOXM1 and CKS1B are downregulated upon silencing of endogenous HPV18 E6/E7 expression in HeLa cells, suggesting that E6/E7 activate FOXM1 and CKS1B expression. This observation is corroborated by qRT-PCR and immunoblot analyses, showing that the knockdown of E6/E7 downregulates FOXM1 and CKS1 on mRNA and protein level in both HPV18- and HPV16-positive cancer cells. Vice versa, E6/E7 overexpression in HPV-negative cells results in FOXM1 and CKS1 upregulation. Using luciferase reporter gene assays, we could determine that the upregulation of FOXM1 and CKS1B occurs via activation of their transcriptional promoters by E6/E7.

Next, the phenotype linked to E6/E7-mediated activation of FOXM1 and CKS1B was analyzed. While FOXM1 silencing per se was not found to have significant effects on the morphology or proliferation of HPV-positive cells, treatment with DNA damaging agents selectively impaired FOXM1-depleted cells in their proliferation and colony formation capacity. This indicates an important role for FOXM1 in DNA damage repair for cervical cancer cells. Further, preliminary results suggest that FOXM1 and CKS1 levels remain elevated in growth-arrested cells under conditions of continuous HPV oncogene expression. This finding is of high interest since FOXM1 expression is generally believed to be strictly proliferation-dependent and sustaining FOXM1 and CKS1 levels may be a mechanism by which E6/E7 increase the therapeutic resistance of non-proliferating HPV-positive cancer cells. In conclusion, two novel cellular target genes of the viral oncoproteins E6 and E7 have been identified. Both FOXM1 and CKS1B are known for their oncogenic potential and support the proliferation of HPV-positive cancer cells. Ongoing studies focus on further elucidating the mechanisms and phenotypic consequences of FOXM1 and CKS1 modulation by E6/E7 which may offer new opportunities for therapeutic intervention.

78

TOPBP1 cooperates with TCOF1/Treacle in the nucleolar response to DNA double-strand breaks

C. Mooser, E. Symeonidou, M. Stucki, D. Fink

Klinik für Gynäkologie, UniversitätsSpital Zurich, Zurich

The DNA damage response (DDR) consists of signalling pathways that are activated in response to DNA damage such as DNA double-strand breaks (DBSs) in order to maintain genomic integrity. The DDR regulates a sophisticated network of cellular activities ranging from DNA repair pathways to transcription. DNA repair in transcriptionally active genomic regions is challenging for the cell and has been associated with ATM-dependent transcriptional repression but the mechanisms involved are still largely elusive. We studied this phenomenon in the rDNA repeats that make up the most actively transcribed regions of the human genome and are localized in the nucleoli, specialized nuclear compartments where ribosome biosynthesis takes place. Here we show that transcriptional repression of rRNA synthesis and nucleolar segregation after rDNA DSBs is dependent on both ATM and ATR activity. Using mainly cell culture and molecular biology-related techniques, transcriptional arrest can be induced by I-Ppo1 expression, leading to the segregation of nucleolar components and nucleolar cap formation. We show protein relocalization of DNA topoisomerase 2-binding protein 1 (TOPBP1) from the nucleoplasm to nucleolar cap during transcriptional inhibition. Mechanistically, TOPBP1 interacts with the C-terminus of TCOF1/Treacle in a phosphorylation-dependent manner through three of its BRCT domains. In summary, our data reveal a novel role of the TOPBP1-TCOF1/Treacle complex in the nucleolar response to DNA damage, where TCOF1/Treacle orchestrates the response to rDNA breaks through the recruitment of the MRN complex and TOPBP1, which lead to ATR activation and transcriptional repression within the nucleoli.

79

Tumor pericytes are immunosuppressive in human non-small cell lung cancer

S. Hall (1), L. Wang (1), C. Simillion (3), P. Dorn (1), S. Berezowska (2), T. Marti (1), R. Peng (1), R. Schmid (1)

(1) Inselspital Bern, University Hospital, Bern, (2) Faculty of Medicine, Institute of Pathology, University of Bern, Bern, (3) Interfaculty Bioinformatics Unit, University of Bern, Bern

Blockade of immune-checkpoint programmed death 1 (PD-1) or PD-1 ligand (PD-L1) using antibodies has shown durable antitumor responses in a minority of patients with advanced non-small cell lung cancer (NSCLC). This suggests the presence of additional immunosuppressive barriers in the tumor microenvironment (TME). Although cancer-associated fibroblasts (CAFs) represent the most prominent cell type in the TME their origin is not known. Lineage tracing data from murines suggest that vascular pericytes are the precursors to CAFs through differentiation and represent an important cellular constituent of the TME. However, in humans our current understanding of the functional role of lung pericytes in lung cancer progression remains largely unknown. Using a flow cytometric based approach, we profiled both lung adenocarcinoma (AC, n = 69) and squamous cell carcinoma (SQCC, n = 66) patient samples and found an enrichment in non-tumor $CD90^+$ mesenchymal cells. Importantly, besides tumor epithelium ($EpCAM^+$), $CD90^+$ mesenchymal cells upregulate PD-L1, which was confirmed histologically. Spatially, $CD90^+$ mesenchymal cells lined blood vessels invading tumor islands, and also were located in the tumor stroma colocalizing with CD73 in both AC and SQCC. $CD90^+$ tumor pericytes prospectively isolated from both AC and SQCC were resistant to the damaging effects of chemotherapy, while priming with INFα and IFNγ induced the upregulation of PD-L1, indoleamine 2,3-dioxygenase (IDO1) and secretion of key cytokines/chemokines critically involved in the functional modification of leukocytes such as TGFβ1. In these same patient samples, we found that the tumor infiltrating $CD8^+$ and $CD4^+$ T cells (TILs) in both AC and SQCC were contained primarily in the memory compartment with an altered differentiation state. Importantly, we were able to identify a combination of compositional features that were predictive of tumor recurrence such as levels of memory $CD4^+PD\text{-}1^+$ TILs. Despite expressing several markers of exhaustion such as PD-1, TIM3 and downregulation CD127, $CD4^+$ and $CD8^+$ effector memory TIL subset maintain an ability to proliferate in culture; however, their function was suppressed in the presence of immune primed $CD90^+$ tumor pericytes. Finally, combined treatment with anti-PD-L1, anti-TGFb1 and IDO1 inhibitor reversed the immunosuppressive properties of $CD90^+$ tumor pericytes on TIL function. In the setting of NSCLC, $CD90^+$ tumor pericytes represent an unrecognized source of chemoresistant and immunosuppressive cells. Our observations suggest that pharmacological modulation of independent biological features present in the non-tumor stroma such as tumor pericytes and their immunosuppressive secretome may be necessary to improve host anti-tumor immunity and patient outcomes in the setting of NSCLC.

80

MDC1 interacts with TOPBP1 to maintain chromosomal stability in mitosis

P. A. Ahorner (1), S. Jones (2), M. De Marco Zompit (1), D. Fink (1), A. Blackford (2), M. Stucki (1)

(1) Klinik für Gynäkologie, UniversitätsSpital Zurich, Zurich, (2) Weatherall Institute of Molecular Medicine, Oxford

MDC1 is a large modular phosphoprotein scaffold that mediates recruitment of signaling and repair complexes to sites of DNA double-strand breaks (DSBs). MDC1 is anchored to damaged chromatin through interaction of its C-terminal BRCT-repeat domain with the tail of gamma-H2AX. It mediates recruitment of several other factors to damaged chromatin via direct phosphorylation-dependent protein-protein interactions. The ubiquitin ligase RNF8 and the structure-specific nuclease complex MRE11-RAD50-NBS1 are among the factors that are recruited to sites of DSBs by direct phosphorylation-dependent interaction with MDC1. Here we introduce a new highly conserved protein interaction surface near the N-terminus of MDC1. Unbiased proteomic analysis revealed that this region in MDC1 constitutes a hitherto unnoticed interaction site for the DNA damage response adaptor protein TOPBP1. We show that through its N-terminal BRCT domains TOPBP1 binds to two conserved Serine residues in MDC1 that are constitutively phosphorylated by caseine kinase 2. Interestingly, disruption of the MDC1-TOPBP1 interaction does not lead to defective recruitment of TOPBP1 to damaged chromatin in interphase cells, where TOPBP1 recruitment is mediated by other factors such as 53BP1. Instead we find that TOPBP1 recruitment to sites of DSBs is dependent on its direct interaction with MDC1 in mitotic chromatin, from which 53BP1 is excluded. Furthermore, we demonstrate that disruption of the MDC1-TOPBP1 interaction renders cells hypersensitive to radiation in mitosis and is associated with increased

micronuclei formation and telomere fragility. Thus, our findings suggest an unexpected and specific role of the MDC1-TOPBP1 complex in the maintenance of genomic stability during mitosis.

81

Exploiting the DNAM-1 system for chimeric antigen receptor (CAR) T cell therapy of glioblastoma

T. Weiss (1), H. Meister (1), M. Weller (1), C. Sentman (2), P. Roth (1)

(1) University Hospital Zurich and University of Zurich, Switzerland, (2) Department of Microbiology & Immunology, Geisel School of Medicine, New Hampshire, USA

Cancer immunotherapy with genetically engineered T cells that express a CAR has led to impressive responses in hematological malignancies and is also explored against glioblastoma, the most common and most aggressive primary brain tumor in adults. However, the immunosuppressive brain tumor microenvironment hampers the anti-tumor efficacy and CAR T cell strategies that are currently being explored against glioblastoma target single tumor antigens, which are non-homogenously expressed and are susceptible to antigen escape.

We addressed these challenges and investigated the DNAM-1 system for CAR T cell therapy. This system comprises the activating receptor DNAX Accessory Molecule-1 (DNAM-1) and the tumor-associated ligands CD155 and CD112.

First, we characterized the target antigens CD155 and CD112 in glioblastoma and demonstrated homogenous expression by flow cytometry and immunohistochemistry of human and mouse glioma cell lines and human glioblastoma tissues. To explore the unknown tumor-intrinsic functional role of these ligands, we generated knockout cell lines using CRISPR/CAS9 and demonstrated that a knockout of CD155 or CD112 impairs the migration and invasion of tumor cells but has no effect on proliferation or sensitivity to chemotherapy or irradiation, which are part of the standard of care for glioblastoma.

Furthermore, we established several DNAM-1-based CAR constructs that use the promiscous binding properties of DNAM-1 and different co-stimulatory domains and investigated their anti-tumor effiacy in vitro and in vivo in fully immunocompetent, orthotopic glioma mouse models. All DNAM-1-based CAR T cell constructs led to high cytolytic activity and effector cytokine secretion in vitro. We demonstrated tumor homing of DNAM-1 CAR T cells upon intravenous administration by in vivo tracking using fluorescence molecular tomography (FMT). CAR T cells transduced with the different DNAM-1-based CAR constructs prolonged the survival of orthotopic glioma-bearing mice upon intravenous and intratumoral administration in two syngeneic models and led to a durable anti-tumor response in a fraction of mice. Their efficacy could be boosted with immune checkpoint inhibition and the treatment was tolerated without toxicities.

Overall, we elucidated the tumor-intrinisic role of CD155 and CD112 and provide a comprehensive systematic preclincal assessment of DNAM-1 CAR T cells against glioma. These findings provide a rationale to test this immunotherapeutic strategy also in human glioma patients.

82

The single cell pathology landscape of breast cancer: tumour cells and their microenvironments

H. W. Jackson (1), J. R. Fischer (1), V. R. T. Zanotelli (1), R. Mechera (2), S. D. Soysal (3), S. Münst Soysal (2), W. P. Weber (4), Z. Varga (3), B. Bodenmiller (1)

(1) Institute of Molecular Life Sciences, University of Zurich, Zurich, (2)Department of Pathology, University Hospital of Basel, Switzerland, (3) Department of Pathology, University Hospital of Zurich, Zurich, Switzerland, (4) Department of Surgery, University Hospital Basel, Switzerland

Single cell analysis has revealed extensive cancer heterogeneity, but histologic stratification of tumors is still the foundation of most clinical decisions. Breast cancer diagnosis based on cellular organization and the expression of hormone receptors, and amplified HER2 has led to subtype targeted therapies and improved patient outcomes, but intra-tumor cellular heterogeneity and the existence of many more molecular subtypes may be responsible for therapeutic resistance and relapse. To extend the histology classification of breast cancer to the single cell level, we applied Imaging Mass Cytometry (IMC) with a 40-parameter antibody panel to create high-dimensional immunohistochemistry pathology images. Single cell segmentation and analysis quantified tumor and stromal single cell phenotypes, their interactions, and spatial heterogeneity in 281 breast cancers. Classification at the cellular level identifies the single cell phenotypes present in hormone receptor positive, HER2 amplified, and triple negative breast cancers as well as the spatial organization of specific tumor microenvironments. Further, classification of breast cancers based on their single cell phenotypes identifies novel subtypes with

distinct clinical outcomes and responses to targeted therapies. Here, single cell pathology provides spatially resolved, multi-cell definitions of breast cancer and identifies cellular targets for future patient-specific therapeutic interventions.

83

Compressive mechanical stress triggers MAPK-dependent loss of cell polarity

R. Mishra, F. Van Drogen, S. S. Lee, P.M. Peter

Department of Biology, Institute of Biochemistry, ETH Zurich

To survive compressive mechanical stress while growing in confined microenvironments or migrating through narrow constrictions, cells reprogram their intracellular organization and acquire appropriate mechanical properties. However, the mechanosensors and downstream signaling networks mediating these changes remain poorly understood. Using a microfluidic device, we recently found that budding yeast sense compressive pressure via the Ca^{2+}-channel Mid1/Cch1 and the cell surface protein Mid2. In turn, Ca^{2+} influx and activation of the Pkc1/Mpk1 MAP kinase pathway lead to depolarization of the actin cytoskeleton, thereby antagonizing polarized growth. We now adapted this platform to study the mechanical response of mammalian epithelial monolayers. Indeed, our preliminary data indicate that like yeast sustained compression of NMuMG E9 cells triggers actin reorganization, loss of cell-cell adhesion and subsequently to Epithelial-Mesenchymal Transition (EMT). Interestingly, ERK5, the Mpk1 homologue, is rapidly activated under these conditions, and ERK5 activity appears to be required for EMT. Moreover, we observe translocation of the transcription factor YAP1 into the nucleus. Taken together, our results suggest that the ERK5-YAP1 signaling network may regulate the cellular response to mechanical stress, and promote EMT and cancer metastasis after growth-induced compression observed in solid tumors.

84

Cytotoxicity of the cucurbitacin E from Citrullus colocynthis (L.) Schrad against multidrug-resistant cancer cells

J. B. Boulos (1), G. E. Elhaboub (2), D. R. Rigano (3), A. S. Saab (4), M. L. Loizzo (5), L. H. Hassan (6), Y. S. Sugimoto (7), R. T. Tundis (5), S. Y. Yagi (2), H. K. Khalid (8), T. Efferth (1), M. Saeed (1)

(1) Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, Germany, (2) Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan, (3) Department of Pharmacy, University Federico II of Naples, Naples, Italy, (4) Faculty of Science II, Department of Biochemistry and Chemistry, Lebanese University, Beirut, Lebanon, (5) Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende (Cosenza), Italy; Department of Biology, University of Pisa, Pisa, Italy, (6) Department of Botany, Faculty of Science & Technology, Omdurman Islamic University, Omdurman, Sudan, (7) Division of Chemotherapy, Faculty of Pharmacy, Keio University, Tokyo, Japan, (8) Department of Pharmacognosy, Faculty of Pharmacy, University of Khartoum, Khartoum, Sudan

Cucurbitacin E (CuE) is an oxygenated tetracyclic triterpenoid isolated from the fruits of Citrullus colocynthis (L.) Schrad. This study outlines CuE's cytotoxic activity against drug-resistant tumor cell lines. Three members of ABC transporters superfamily, P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and ABCB5, whose overexpression in tumors tightly linked to multidrug resistance, were investigated. Further factors contributing to drug resistance studied were the tumor suppressor TP53 and the epidermal growth factor receptor (EGFR). Multidrug-resistant cells overexpressing P-gp or BCRP were cross-resistant to CuE. By contrast, ABCB5-overexpressing cells and TP53 knock- out cells were sensitive to CuE. Remarkably, resistant cells transfected with oncogenic ΔEGFR were hypersensitive (collateral sensitive) to CuE. In silico analyses demonstrated that CuE is a substrate for P-gp and BCRP. Immunoblotting analyses highlighted that CuE targets EGFR and silenced its downstream signaling cascades. The COMPARE analyses of transcriptome-wide expression profiles of tumor cell lines of the NCI identified common genes involved in cell cycle regulation, cellular adhesion and intracellular communication for different cucurbitacins. In conclusion, CuE represents a potential therapeutic candidate for the treatment of some types of refractory tumors.

85

PTEN loss in non-small cell lung cancer: Effect on immune cells

U. Rulle, C. Wu, R. Casanova, A. L. Leblond, A. Soltermann

Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich

Introduction: PTEN (phosphatase and tensin homolog) is known to be a major tumour suppressor, and may serve as biomarker for a hyperactivated PI3K-Akt pathway. This leads to oncologic rationale that PTEN loss might indicate better response to PI3K inhibitors such as Pictilisib. Other researchers showed that in melanomas PTEN loss can indicate immunosuppressive microenvironment. In our previous study we found that in non-small cell lung cancer (NSCLC) PTEN loss is up to 50%. In continue of that study we further investigate PTEN loss effect on immune cells in NSCLC.

Methods: In order to understand PTEN interaction with immune cells we used TMA with 1109 cases - 378 lung squamous carcinomas (LSCC), 497 adenocarcinoma (LADC), of which 875 chemo-naïve and 105 chemo-treated. Staining and scoring was done for PTEN, PD-L1, CD8 markers as well as we scored lymphocytes according to cell shape using computer-based method.

Results: Results indicated inverse correlation between PTEN and PDL1 -0.202 ($p<0.001$) in all chemo naïve NSCLC and -0.224 ($p=0.009$) chemo treated. PDL1 also had a positive correlation with CD8 0.141 ($p<0.001$) in chemo naive cases. Inverse correlation between PTEN and PDL1 was stronger in LSCC. Lymphocyte score from computer based method correlated with PTEN 0.120 ($p=0.023$) and PDL1 0.119 ($p=0.005$) in LSCC and in LADC with PTEN 0.131 ($p=0.005$).

Conclusion: In our cohort we found out that PTEN loss associated with higher PDL1 expression in both chemo-naïve and chemo-treated NSCLC with higher correlation in LSCC. As well as lymphocyte score correlated with PTEN and PDL1. We can conclude that there is some connection between PTEN and immune cells, but further investigation is still needed.

86

Targeting Fibroblast Growth Factor Receptors in Rhabdomyosarcoma

N. A. Alijaj (1), S. A. Moutel (2), M. A. Gray (1), M. R. Roveri (1), G. A. Manzella (1), M. C. Wachtel (1), F. R. Perez (2), B. E. Schäfer (1), M. I. Bernasconi (1)

(1) Oncology, University Children's Hospital, Zurich, (2) Institut Curie, PSL Research University, Paris

Rhabdomyosarcoma (RMS) is an aggressive pediatric soft tissue sarcoma and is classified into two main histopathological subtypes: embryonal RMS (eRMS), characterized by different genomic changes or alveolar RMS (aRMS), driven by the oncogenic fusion protein PAX3-FOXO1. The significant toxicity associated with conventional chemotherapies represents a major complication in pediatric oncology. To improve current therapies, we adopted two different strategies targeting fibroblast growth factor receptors (FGFR) in RMS.

FGFR1-4 are a family of transmembrane receptor tyrosine kinases. Their activation upon binding of fibroblast growth factors (FGF) triggers pro-survival and proliferative signals.

Our goal is to deliver drugs specifically to the tumor site by taking advantage of FGFR4 overexpression in RMS. To this end, we will covalently link FGFR4 specific nanobodies to the surface of liposomal vincristine in order to actively target RMS cells. We have established the optimal conditions to formulate liposomes loaded with vincristine. Following nanobody phage display selection on recombinant FGFR4 we focused on the top scoring candidates. Flow cytometry analysis on FGFR4-expressing versus FGFR4 knock-out RMS cell lines showed receptor-specific binding of three nanobodies. In activation assays with the FGFR4 specific growth factor FGF19, we demonstrated that the three binding candidates also blocked downstream ERK activation in RMS cells. We will now assess their theranostic potential on drug-loaded and fluorescently labeled nanovesicles on RMS tumor cells in vitro and in xenografts in vivo.

Surprisingly, we observed change in cell morphology followed by cell death upon exposure to FGF2 in a subset of cultured cells established from eRMS patient-derived xenografts. Inappropriate expression of FGFRs and FGF signaling is implicated in tumor progression and therefore our findings appear contradictory. Dose-response experiments have shown that FGFR inhibition with small molecule inhibitors completely rescued FGF2 toxicity. In contrast, however, we detected high expression levels of FGFR1, 2 and 4 as well as activating mutations of FGFR4 in FGF2-sensitive eRMS cells. Therefore, our results are of

upmost clinical relevance since genetically-based drug selection could lead to an inappropriate treatment inducing tumor promoting conditions. Hence, our second goal is to further unravel the molecular mechanism underlying the toxic effect of FGF-2 in a subset of eRMS tumors to avoid potentially harmful treatments.
In summary, we have identified FGFR4 specific nanobodies that bind to the receptor and block downstream signaling in RMS cells. Active drug delivery of liposomal vincristine to the tumor site has the potential to enhance the therapeutic impact and decrease side effects. Moreover, we discovered a toxic effect of FGF2 in a subgroup of eRMS patient derived xenograft cells which might open new avenues for treatment.

87

Interplay between epithelial-mesenchymal transition and reactive oxygen species

A. Morand, F. Tang, S. Piller, G. M. Christofori

Department of Biomedicine University of Basel, Switzerland

Although therapies have advanced the clinical outcome of cancer patients, metastatic cancer remains a deadly disease. While evidences suggest a role of an epithelial-mesenchymal transition (EMT) program in metastasis formation and resistance, the mechanism remains obscure. Tumor microenvironment critically influences malignant tumor progression and can induce an EMT, which is coupled with toxic reactive oxygen species (ROS). However, the interplay between ROS and an EMT is hardly understood.
In order to explore the crosstalk between ROS and an EMT, epithelial Py2T murine breast cancer cells were treated with TGFb supplemented by the N-acetyl-cysteine (NAC) antioxidant. Moreover, we have investigated the effect of H2O2 on an EMT. While the antioxidant treatment sufficiently prevents a TGFb-induced EMT of epithelial Py2T cells, NAC has little effect on reverting mesenchymal long-term TGFb-treated Py2T (Py2T LT) cells. Moreover, we have found that long-term exposure of H2O2 promotes an EMT progression in epithelial Py2T cells (H2O2 Py2T) in vitro. Also, after injection of the H2O2 Py2T cells into the fat pad of NOD scid gamma (NSG) mice, we have observed a high rate of metastasis compare to TGFb-mesenchymal Py2T LT cells, suggesting a role of oxidative stress in metastatic progression.
Taken together, our study suggests a crucial role of ROS in the initiation/early stages of an EMT and it demonstrates that increased ROS stress can drive an EMT and metastatic progression.

88

Transcriptomic profiling reveals dynamic functional remodeling of the bone marrow microenvironment during postnatal development, aging, and inflammation

P. M. Helbling (1), E. Pineiro (2), R. Gerosa (1), S. Böttcher (1), F. Al-Sharour (2), M. G. Manz (1), C. Nombela-Arrieta (1)

(1) Department of Hematology and Oncology, University Hospital Zurich, Zurich, (2) Bioinformatics Unit, Spanish National Cancer Research Center, Madrid

Stromal components are not only fundamental structural constituents of the bone marrow (BM), but they also provide extrinsic cues to critically regulate the hematopoietic system and support its maintenance and adaptation to external stress. Vital stromal-hematopoietic interactions in defined niches regulate many levels of hematopoiesis, from hematopoietic stem cell (HSC) maintenance, to lineage differentiation and maturation. The identity and function of hematopoiesis-regulating BM stromal cells has been best studied in homeostasis. Nonetheless, the dynamic changes in composition, function, and molecular identity that BM stromal cells undergo during homeostatic aging as well as in inflammatory settings, and how these relate to alterations in hematopoietic output, are poorly understood.
We performed a transcriptome-wide gene expression analysis (RNA-seq) of four murine mesenchymal and vascular BM cell populations, which together comprise the majority of the known medullary stroma, namely CXCL12-abundant reticular cells (CARc), PαS cells, sinusoidal endothelial cells (SECs), and arterial endothelial cells (AECs). To obtain a comprehensive view of stromal adaptations during the normal life span and during inflammation, we profiled cells isolated from 2 weeks, 2 months and 2 years old mice, as well as mice stimulated with bacterial and viral infection-mimicking agents (LPS and poly I:C). Using this global dataset, we report that: i) molecular fingerprints inform on potentially novel stromal cell-specific functional features and define identity markers for the investigation of topographical distribution and cellular interactions in situii) major remodeling of the transcriptional landscape occurs in the transition from post-natal to adult period, when HSC proliferation is known to drop sharply iii) aging leads to a predominant upregulation of pro-inflammatory gene expression iv) inflammatory stimuli trigger massive and pathogen-specific adaptations in the transcriptome of all cell types analyzed v) convergent gene-expression programs exist in aging and inflammation, which partially explain aging-driven alterations in BM hematopoietic function.

89

Effective priming of CD39+ PDhigh CD8+ T cells by the primary tumor mediates metastatic dormancy in breast cancer

P. Tallón de Lara (1), M. Marijne (1), N. Núñez (1), H. Castañón Cuadrado (1), V. Cecconi (1), F. Movahedian Attar (1), S. Hiltbrunner (2), I. Glarner (1), E. Orlando (3), F. Uliana (3), R. Aebersold (3), S. Tugues Solsona (1), B. Becher (1), M. Van den Broek (1)

(1) Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland, (2) Deparment of Oncology and Hematology, Zurich University Hospital, Zurich, (3) Institute of Molecular Systems Biology ETH Zurich, Switzerland

Although metastatic disease can be present already at diagnosis, in some patients it appears months or years after resection of the primary tumor. This is thought to be due to the ability of some tumor cells to remain dormant after metastatic seeding. It is not known how dormancy is controlled, but there is evidence for tumor cell-intrinsic and -extrinsic mechanisms including immune defense.

To investigate whether and how immune defense controls metastasis to the lungs, we used two cell lines – 4T1 and 4T07 – derived from the same spontaneous breast tumor in immunocompetent BALB/c mice. We detected 4T1 and 4T07 cells in the blood and lungs of tumor-bearing mice, suggesting that both cell lines can disseminate from a primary tumor and seed the lungs. However, only orthotopic 4T1 tumors gave rise to progressive metastatic disease in the lungs, whereas orthotopic 4T07 tumors did not. Thus, disseminated 4T07 cells displayed a dormant behavior.

Dormancy of 4T07 cells in the lungs was completely dependent on the generation of systemic immunity by the primary tumor. Indeed, intravenously injected 4T07 cells aggressively metastasized to the lungs in tumor-naive animals but fail to form macrometastasis in animals bearing a primary 4T07 tumor. Specifically, we identified CD8+ T-cells as essential for 4T07 dormancy. High-dimensional flow cytometry analysis showed that $CD8^{+}$ PD^{high} $CD39^{+}$ T cells heavily infiltrated 4T07 primary tumors, whereas they were virtually absent form 4T1 tumors.

Together, our data suggest that 4T07 tumors induce systemic immunity that induces metastatic dormancy. Absence of this protective response in mice with 4T1 tumors allows the development of metastasis. Secretome analysis detected many difference between 4T1 and 4T07 cells. We currently focus on selected candidates and investigate their ability to interfere with priming of protective, CD8+ T-cell-dependent immunity.

90

New Regulators of the Notch pathway

D. Kantar, L. Héron-Milhavet, A. Djiane

Institut de recherche en cancérologie de Montpellier

The team is interested in understanding the mechanisms by which Notch signalling triggers epithelial tissue growth. Using unique highly tractable genetic models and combining genome-wide analyses (RNA-Seq and ChIP-on-chip) the lab identified the direct Notch targets during hyperplastic growth (activated Notch), dysplastic growth (scribble mutants) and neoplastic growth (the combination of the two). Further differential expression analyses highlighted a neoplastic specific program.

Many of these drosophila genes found have clear human orthologues that could help us understand the progression of cancer cells from hyperplastic to neoplastic growth and that could therefore represent new potential tumour suppressors or oncogenes in the context of activated Notch. My PhD project is to study the role of a new Notch pathway regulator: mxc/NPAT.

Our studies in Drosophila revealed important fluctuations of Histone levels and of several genes controlling the amount of Histone loci transcription, between hyperplasia and neoplasia, suggesting that Histone regulation and the transcriptional machinery regulating Histone genes expression could cooperate with Notch to command specific transcriptional programs. Therefore, I will study the role of NPAT, a histone transcription activator, on the acquisition of an invasive behaviour in Notch addicted cell lines and its effect on the Notch pathway components.

Our results suggest that NPAT, in addition to its role in regulating histones genes transcription, affects the localization of the Notch1 receptor in the cell, is able to change the activity of this pathway by affecting the levels of the Nicd (Notch Intracellular Domain) and in consequence the expression levels of its target genes.

91

The impact of hyperthermic intraperitoneal chemotherapy (HIPEC) on the anticancer immune response

L. Roth, E. Breuer, R. Graf, A. Gupta, P. A. Clavien

Department of Surgery and Transplantation, University Hospital Zurich, Switzerland

Background: The outcome of peritoneal carcinomatosis, often occurring due to appendix or colorectal cancer, has dramatically improved with the combination of cytoreductive surgery (CRS) and hyperthermic (430C) intraperitoneal chemotherapy (HIPEC). Nevertheless, recurrence of the disease presumably due to remnant cancer cells limits survival of the patients, suggesting further optimization in HIPEC treatment. Therefore, it is important to understand mechanisms operating behind HIPEC treatment. Since chemotherapy may induce immunogenic changes, we analyzed effects of MitomycinC/Doxorubicin, widely used chemotherapeutics in HIPEC settings, on the immunogenicity of colorectal cancer cells in-vitro. We examined expression of immunogenic cancer-testis antigens (CTA) on cancer cells after HIPEC like conditions in-vitro and subsequent dendritic cell (DC) maturation and cytotoxic T-cell activation.

Methods: Multiple colorectal cancer cell-lines were treated with MitomycinC/Doxorubicin for 30 minutes with and without hyperthermia (430C). After the treatment, cells were further incubated at 370C for 72 hours. After 72 hours, CTA expression was analyzed using qPCR and western blot. To assess DC maturation, we set up a co-culture between differentially treated colorectal cells and monocyte-derived DC`s. We analyzed surface markers such as HLA-DR, CD 86 and CD 83 to assess DC maturation using flow cytometry. Further, the activation of cytotoxic T-cells was measured by intracellular IFN-y staining after co-culture with DC`s that were pre-incubated with treated and untreated colorectal cancer cells.

Results: Initial qPCR screening of CTA revealed that two CTA namely, Cyclin A1 and SSX-4 were upregulated after HIPEC treatment. Compared to the control condition (no drug, 370C), MitomycinC/Doxorubicin in HIPEC condition led to an increase of Cyclin A1 up to 53 folds and SSX-4 to 30 folds. The amount of Cyclin A1 protein was doubled compared to the control treatment (p=0.0015, CI mean volume intensity 0.1989 – 0.4233). After co-culturing with HIPEC treated colorectal cancer cells, we noticed significant expression in CD 83, a DC activation marker. DCs that were activated upon incubation with HIPEC-treated cancer cells were able to prime cytotoxic T-cells leading to enhanced IFN-y production by the CD8+ T cells.

Conclusions: HIPEC treatment can cause immunogenic changes in colorectal cancer cells. This is supported by the upregulation of certain CT-antigens with HIPEC treatment. Changes of cancer cells induced by HIPEC lead to DC activation and subsequent cytotoxic T-cell activation. This could explain a part of the mechanism, how HIPEC treatment may work and inclusion of immunotherapy may improve outcome of this treatment.

92

HOXA13 drives hepatocytes proliferation and liver tumorigenesis in mice

G. Bianco (1), H. Montazeri (1), L. Quagliata (1), T. O'Connor (2), U. Ehmer (3), R. Oellinger (3), M. Matter (1), G. Christofori (4), C. K. Y. Ng (1), S. Piscuoglio (1), M. Heikenwaelder (5), L. M. Terracciano (1)

(1) University Hospital, Basel, (2) Helmholtz Zentrum, Munich, Germany, (3) Technische Universitaet Muenchen (TUM), Munich, Germany, (4) Department of Biomedicine University of Basel, Switzerland, (5) Deutsches Krebsforschungzentrum (DKFZ), Heidelberg, Germany

Introduction: Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and the third most common cause of cancer-related mortality worldwide. For patients suffering from advanced-stage disease, the few therapeutic options available are not curative and improve patient survival by only a few months. Therefore, new molecular targets that can be explored as therapeutic options are highly needed. Class I Homeobox (HOX) genes are fundamental components of embryonic patterning and morphogenesis, with expression persisting into adulthood. They are also implicated in neoplastic transformations. However, the role of HOX genes is poorly understood and the functional relationship between the malignant phenotype and abnormal expression of HOX genes is still unclear. In this study we sought to define the role of the HOXA13 gene in hepato-carcinogenesis using in vivo models.

Methods: To unravel the molecular mechanism of HOXA13 driven tumorigenesis in liver and its direct oncogenicity in vivo, a murine model of HOXA13 overexpression in liver was generated using hydrodynamic injection coupled with a transposase system. This model led to the stable and specific HOXA13 expression in C57BL6\J mouse

hepatocytes up to 5 months post injection. Mouse phenotype was followed over time, from 2 weeks up to 1-year post injection. 16 mice (8 for CTRL vector and 8 for HOXA13) were injected and sacrificed for every time point. RNA-sequencing was performed to monitor the transcriptomic changes over time.

Results: 1-year post-injection 50% (4/8) of the injected mice with HOXA13 developed liver tumors of various histological grades and types, from very well differentiated HCCs to very highly undifferentiated and cholangiocarcinoma-like nodules. HOXA13 overexpression in the liver led to highly proliferative hepatocytes after only 2 weeks and the proliferative phenotype was maintained until 5 months post-injection, when pre-neoplastic lesions began to form. HOXA13 overexpression correlated not only with proliferation but also with the DNA damage marker yH2AX, suggesting a possible mechanism of tumorigenesis driven by genome instability. Gene set enrichment analysis of RNA-seq performed on whole liver extracts of 2 week-old mice and tumors showed that the main pathways involved in HOXA13 expression are cell cycle, in particular G2/M transition and mitotic assembly checkpoint, angiogenesis, TP53 pathway, IL6-JAK-STAT3 signaling, Notch signaling and epithelial to mesenchymal transition.

Conclusion: Our study highlights the key role of HOXA13 as a potential novel oncogene in HCC development and suggests possible mechanisms through which it drives liver tumorigenesis. We expect that the generated data in vivo, coupled with mass spectrometry and ChIP-sequencing experiments performed in vitro, will further help us identify down-stream effectors of HOXA13 thus providing new potential therapeutic targets for HCC.

93

Microfluidic-based immunohistochemistry combined with next generation sequencing on diagnostic tissue sections for detection of tumoral BRAF mutation

A. L. Leblond (1), M. Rechsteiner (1), A. Jones (2), S. Brajkovic (2), D. Dupouy (2), A. Soltermann (1)

(1) Institute of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland, (2) Lunaphore Technologies SA, EPFL Innovation Park, Building C, CH-1015, Lausanne, Switzerland

Objectives: Tailored diagnostic requires sequential combination of assays, including immunohistochemistry (IHC) and next generation sequencing (NGS). Here we combined concurrently on a single formalin-fixed paraffin-embedded (FFPE) slide microfluidic-based IHC (micro-IHC) and NGS for BRAF V600E mutation detection.

Methods: For micro-IHC, we carried out only the primary antibody incubation step of the conventional chromogenic IHC on the LABSAT device developed by Lunaphore Technologies SA. We stained different cancer tissues for pan-cytokeratin (pan-CK) and further investigated them for H score and NGS for BRAF V600E mutation.

Results: Micro-IHC led to robust immunoreactivity that varied exponentially within a couple of minutes and performed similarly to conventional automated IHC. BRAF V600E mutation was detected by NGS using genomic DNA extracted from the pan-CK positive areas.

Conclusions: We showed that micro-IHC is a reliable approach to combine minute range IHC and molecular analysis by NGS on the same FFPE tissue section.

94

Modeling of prostate cancer heterogeneity with organoid technology to dissect TIP5 function in prostate cancer

K. N. Pietrzak, D. Bär, R. Santoro

Department of Molecular Mechanisms of Disease, University of Zurich, Zurich, Switzerland

Prostate cancer (PCa) is the second leading cause of cancer death in men. The elusive mechanisms underlying PCa development, its heterogenous molecular landscape, lack of early prognostic markers and efficient therapies for advanced PCa remain major clinical concerns. We have recently shown that the epigenetic factor TIP5 is implicated in aggressive PCa (Gu et al., 2015). To determine how TIP5 affects PCa initiation and development, we analyzed the contribution of TIP5 in prostate epithelium. Analysis of prostate duct structure of 6 months old Tip5-KO mice revealed structural alterations such as increased size and thinner and ragged luminal epithelium layer, suggesting defects in prostate epithelium maintenance. Gene expression analysis of basal and luminal prostate epithelium cells isolated from Tip5 KO mice indicates that TIP5 plays a major role in luminal cells, which are considered the cell of origin of aggressive PCa. These results were further supported by the analysis of prostate organoids. In the absence of TIP5, the capacity of luminal prostate progenitors to form

organoids was compromised, suggesting a role of TIP5 in luminal progenitor cells. To determine the role of TIP5 in PCa, we established PCa organoid models that take into consideration the cell of origin, oncogenic insult and the order of oncogenic events. As a proof of concept, we generated organoids derived from basal or luminal prostate epithelial cells depleted of PTEN (Pten KD), which is a tumor suppressor gene commonly mutated in human PCa and whose mutations correlate with higher TIP5 expression in PCa patients. Phenotypic and transcriptomic analyses indicated on oncogenic transformation of Pten KD organoids. Remarkably, depletion of Tip5 before induction of Pten downregulation impaired Pten loss-mediated oncogenic transformation particularly in the case of organoids of luminal origin. Surprisingly, the change of the order of events, where Pten downregulation was achieved before Tip5 depletion gave rise to PCa organoids. Taken together, these results suggest that TIP5 is required for the loss-of-Pten mediated initiation of luminal origin PCa and the cell type together with the order of oncogenic events are critical determinants in PCa development.

95

Targeting of TIP5 bromodomain has a regulatory impact of stem cell-like features in prostate cancer progenitor cells

R. Peña-Hernández, S. Frommel, D. Bär, R. Santoro

Department of Molecular Mechanisms of Disease, University of Zurich, Zurich, Switzerland

Prostate cancer (PCa) is one of the most prevalent cancers in men. It has an unpredictable clinical history; while most tumors are indolent, some patients display lethal phenotypes. Treatment of metastatic disease is not curative and leads to acquire resistance and relapse. Recently, we showed that TIP5 is highly expressed in metastatic PCa and establishes epigenetic silencing of genes commonly repressed in metastatic tumors (Gu et al. 2015, Nature Genetics). TIP5 is required for PCa cell proliferation, invasion and regulation of developmental genes in tumors with high TIP5 levels, displaying a dedifferentiated phenotype. Cancer stem cells are a rare cell population with unlimited proliferative, multi-potency, renewal, and highly tumorigenic capacities, representing an important target against aggressive PCa. To determine whether TIP5 is a therapeutic target in metastatic PCa, we established a culture condition allowing the isolation of PCa progenitors cells with stem features from the metastatic PCa PC3 cell line. RNAseq analysis revealed that PCa progenitors resemble stemness features such as downregulation of developmental genes and upregulation of CSC markers such as LGR5, CD24 and ALDH1A2. Remarkably, TIP5 knockdown inhibits PCa stem cells. We identify TIP5-BRD as an epigenetic reader of H3K14ac. Expression of TIP5-BRD mutants with impaired ability to recognize H3K14ac compromised the growth of PCa progenitors. Moreover, treatment with TIP5-BRD inhibitors specifically impairs the growth of PCa stem cells without affecting proliferation of the total population of PC3 cells. We found that a subset of genes downregulated in PCa progenitors compared to PC3 cells, are characterized by a bivalent epigenetic signature and enrichment in H3K14ac, suggesting a possible role of TIP5 in the regulation of these genes. These findings evidence the role of TIP5 in the modulation of PCa stem cell features and that TIP5 is a potential therapeutic target for aggressive PCa.

96

A multi-step framework to analyse high-throughput drug combination screens

P. Jaaks (1), D. J. Vis (2), E. Coker (1), N. Aben (2), A. S. Barthorpe (1), D. Van der Meer (1), H. Lightfoot (1), M. Michaut (2), R. Rahman (2), L. Wessels (2), M. J. Garnett (1)

(1) Translational Cancer Genomics, Wellcome Sanger Institute, Hinxton, United Kingdom, (2) Division of Molecular Carcinogenesis, The Netherlands Cancer Institute, Amsterdam, Netherlands

Introduction: Tailored, genotype-driven therapies are the future for treatment of heterogeneous cancers like breast cancer. However, despite their high therapeutic index, targeted agents often have limited success in single agent treatment setups due to variable clinical response rates and resistance. Thus, a framework to develop drug combinations for treatment of cancer is needed.

Material and methods: Our group has an ongoing effort to systematically screen two-drug combinations in cancer type-specific and pan-cancer high-throughput drug combination screens. We have screened over 1000 combinations, including both broad-acting chemotherapeutics and targeted compounds. Cancer type-specific drug combination screens were conducted in breast and colorectal cancer cell lines, using a panel of 50 cell lines in each screen. Acquired data has been used to determine a strategy to extract the most promising drug combinations in each tissue.

Results and discussion: We propose a multi-step analytical framework for the identification and prioritisation of synergistic combinations. Initial filtering is based on several parameters, including synergy (z-score), XMID and Emax. Robustness of drug response effects is assessed through rescreening of selected drug combinations. Further prioritisation of promising combinations in a tissue-specific setting is achieved through stratification of the combination drug response in cell line subgroups, including segregation based on commonly mutated genes and molecular subtypes (e.g. PAM50 subgrouping in breast cancer). Moreover, systematic exploration of genotype-drug synergy associations will be used to identify biomarkers of response for patient stratification.

Conclusion: In conclusion, we have developed a multi-step framework to identify clinically relevant synergistic drug combinations complementing current cancer therapy options. This pipeline may facilitate future combination drug synergy predictions and validation.

97

The role of the miR-143/miR-145 cluster in chondrosarcoma progression

J. Urdinez (2), A. Boro (1), J. Snedeker (2), A. Gvozdenovic (2)

(1) University Hospital Balgrist, University of Zurich, Zurich, (2) University Hospital Balgrist, University of Zurich / Institute for Biomechanics, ETH Zurich, Zurich

Chondrosarcoma (CS) is the second most frequent bone sarcoma. Due to the inherent chemo- and radiotherapy resistance and absence of known therapeutic targets, clinical management is limited to surgical resection and consequently patients with advanced disease face a poor prognosis. Hence, there is an urgent need for identification of genetic drivers in CS in order to develop effective and specific therapeutic strategies.

With initial focus on down-regulated miRNAs, we performed a small-RNA sequencing study in chondrogenic tumor samples which revealed that miRNA levels of the cluster miR-143/miR-145 inversely correlated with tumor malignancy. miRNA-143/145 levels were likewise downregulated in plasma samples of patients with high grade CS compared to those with low grade CS. Deregulation of both miRNAs in tumors and plasma was subsequently confirmed in a validation cohort. Interestingly, the artificial upregulation of miR-143/145 suppressed the in vitro ability of CS cells to form colonies in soft agar without affecting their migratory and proliferative capacity. Additionally, the overexpression caused a G1 arrest in the cell cycle as assessed by flow cytometry and a changed affinity to adhere to extracellular matrix substrates abundant in the primary tumor site. Importantly, cluster overexpression significantly reduced primary tumor growth in an in vivo orthotopic xenograft model in SCID mice.

To elucidate the underlying mechanisms of the tumor-suppressive effects of miR-143/145 cluster, we developed a combinatorial analysis strategy to identify putative miR-143/145-regulated gene targets. We identified FSCN1 as potential miR-143/145 target in CS through combining in silico prediction tools with mRNA expression profiles obtained by sequencing. FSCN1 mRNA and protein levels were significantly downregulated in samples overexpressing miR-143/145. Furthermore, a luciferase assay confirmed that FSCN1 is a direct target of both miRNAs. Mutagenesis of miRNA binding sites within the FSCN1 3'-UTR region abolished the inhibitory effect of miR-143 and miR-145 on the luciferase activity. Additionally, immunostaining of a CS tissue microarray revealed that the number of samples positively stained for FSCN1 increased with tumor grade. Lastly, by knocking down FSCN1 in CS cells we could demonstrate using different in vitro assays that this gene contributes to the oncogenic potential of tumors.

The first reported miRNA sequencing study shows a robust approach for the discovery of CS drivers based on the initial identification of aberrantly expressed miRNAs. We demonstrated that miR-143/145 cluster plays a tumor-suppressor role in CS. Moreover, we identified FSCN1 as a direct target of miR-143/145 and as a novel potential therapeutic target in CS.

98

Molecular and functional characterization of papillary renal cell carcinoma (pRCC) type 2

S. Angori (1), P. Schraml (1), H. Moch (1), A. Kahraman (1), R. Ohashi (2)

(1) Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, (2) Histopathology Core Facility, Niigata University Faculty of Medicine, Japan

Introduction: Renal cell carcinoma (RCC) refers to a heterogeneous group of cancers derived from renal tubular cells. Papillary renal cell carcinoma (pRCC) represents the second most common histologic subtype, accounting for about 15% of all renal cell cancers. pRCC is further subdivided into type 1 with single layered small cell and scanty

cytoplasm and type 2 with large pseudostratified cells and eosinophilic cytoplasm. pRCC type 2 are more often associated with a higher tumor stage and grade, higher frequency of necrosis and sarcomatoid features with worse and more aggressive outcome. pRCC type 1 is characterized by chromosome 7 gains and mutations in the oncogene MET, while recent data indicated that pRCC type 2 consists of several subgroups characterized by genome stability (with only few chromosomal aberrations) and instability (with many chromosomal alterations, mainly deletions). Based on this molecular background, the aim of the project is a detailed molecular characterization of pRCC type 2 to uncover potential new molecular subgroups.

Methods: Comprehensive molecular characterization of 60 formalin-fixed, paraffin-embedded tissue of pRCC type 2 tumor samples is performed using a copy number variations analysis with the OncoScan assay. In this analysis, thousands of probes map to a specific location on a genome, allowing to visualize copy number changes in each chromosome. A cohort of 13 pRCC type 1 patients is used as a control. pRCC samples have been selected according to a percentage of tumor cells higher than 45% using the Nexus Express Software for OncoScan. Whole Exome Sequencing (WES) will be used to detect gene mutations that point to tumor promoting pathways affected in pRCC type 2. Functional assays using patient-derived primary cell cultures will be applied to validate WES data. Due the lack of pRCC cell line, we decided to establish a patient-derived primary cell culture model. Fibroblast reduction, increase of the cell growth rate and the match between the primary tumor and the cell culture molecular profiles are the main challenging points for optimizing the cell culture protocol. In order to select a pure tumor cell culture, different strategies have been tested as seeding of the cells in ultra-low binding plates, different culture media and different trypsinization times.

Results: The copy number variation profiles of pRCC samples are defined by multiple chromosomal gains. pRCC type 1 show more copy number gains in chromosome 7 (92%) and 17 (100%) than in pRCC type 2 (74% in both chromosomes). Chromosome 16 gain, however, is more predominant in pRCC type 2 (74%), than in pRCC type 1 (38%). A chromosome gain is also common in chromosome 3 (29% pRCC type 2, 15% pRCC type 1), in chromosome 12 (34% pRCC type 2, 15% pRCC type 1) and in chromosome 20 (43% pRCC type 2, 23% pRCC type 1). A copy number loss is identified only in chromosome X (62% of pRCC type 1 and 46% pRCC type 2). After establishing the protocol for a pure primary cell culture, c-MET amplification and IHC of kidney markers can be tested to prove that the mutation profile of the primary cell culture resembles the pRCC type 1 primary tumor.

Conclusion: Type 1 and type 2 pRCC are shown to have a similar CNV profile, including gains in chromosome 3, 7, 12, 16, 17 and 20 and a loss in chromosome X. The combination of CNV analysis, WES data and the functional assays will help to better the biology of this disease leading to a more appropriate clinical management of these pRCC patients.

99

Characterisation of Renal Cell Carcinomas with wtVHL Clear Cell RCC as a Focal Point

A. A. Batavia (2), J. Kuipers (2), P. Schraml (1), N. Beerenwinkel (2), H. Moch (1)

(1) Institute of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland, (2) Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

Molecular characterisation of cancers is central to precision medicine. This is vital for Renal Cell Carcinomas (RCC) where traditional therapies such as chemotherapy are not effective. Stratifying tumours on the bases of their detailed molecular profiles would yield new and more effective targets for RCC treatment. Clear cell renal cell carcinomas (ccRCC) are by far the most common forms of kidney cancer. The majority of ccRCC tumours are characterised by the biallelic inactivation of the VHL tumour suppressor gene. However, in approximately 5-12% of cases tumorigenesis occurs despite the presence of active VHL. Tumours with active VHL have been shown to be more aggressive than then those with VHL inactivation with patients having a worse prognosis.

A multidisciplinary approach is used to determine the possible cause and route for $_{wt}$VHL ccRCC development. Samples are gathered from both publically available datasets (e.g. TCGA) and the USZ biobank. VHL can be inactivated via three methods: mutation, chromosome 3p deletion and promotor hypermethylation each of which are assessed to determine VHL status. Sanger sequencing of VHL is routinely carried out for all ccRCC samples frRm the USZ biobank. FFPE extracted DNA from samples in which no VHL mutations were found underwent OncoScan analysis and bisulfite sequencing to assess somatic copy number variations and promoter hypermethylation respectively. Of the 99 samples sent for OncoScan, 12 had their 3p regions intact with an additional 5 with a CN-LOH. 4 of these 12 had chromosome 8q loss suggesting these tumours may belong to the rare TCEB1 RCC subtype. Cases with $_{wt}$VHL sequence with their 3p arm intact were associated

with amplifications in chromosome 9p, 13 and regions within chromosome 1p. Cases with 3p arm deletion were associated with amplifications in the 5q region and deletions in chromosome 14q. Bisulfite sequences of 85 of the 99 samples have been analysed thus far. 12 samples with $\neg_{wt}$VHL sequence and with chromosome 3p intact (including all but one CN-LOH cases) were non-methylated. 27 of the samples have non-methylated promoters with 3p deletion indicating a single allelic inactivation of VHL.

In parallel, $_{wt}$VHL ccRCC samples were identified from the 242 ccRCC samples in TCGA with mutation, copy number and methylation data available. 10 samples were found to have fully active VHL and 86 samples have a $_{wt}$VHL sequence with a single allelic inactivation via 3p deletion or hypermethylation. Using the clinical data available for these patients, we found those with biallelic activation of VHL to have the worst survival. Those with a single allelic inactivation also showed a trend toward a lower survival rate compared to those with biallelic inactivation.

Overall, we have 12 and 10 patients in our two cohorts with an additional 27 and 86 with a single active copy of VHL. Due to the small sample sizes, a top down approach will be taken in order to reliably identify the molecular characteristics of biallelic activated VHL tumours. Using pan-kidney and pan-cancer cohorts, the locations of biallelic activated VHL tumours in relation to other tumour types in a multi-dimensional space created using 'omics data would help reveal which tumours these are closely relate to. This by extension will reveal the key features distinguishing biallelic activated VHL tumours from biallelic inactivated VHL ccRCC tumours.

100

Cancer cell-intrinsic cGAS expression mediates tumor immunogenicity

L. Schadt (1), C. Sparano (1), N. A. Schweiger (1), K. Silina (1), V. Cecconi (1), E. Guggisberg (1), S. Saba (1), A. Weber (2), H. Moch (2), W. Barchet (3), M. Van den Broek (1)

(1) Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland, (2) Institute of Pathology and Molecular Pathology, University Hospital Zurich and University of Zurich, Zurich, Switzerland, (3) Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, University of Bonn, Germany

Tumor immunogenicity is a prerequisite for spontaneous antitumor T-cell immunity, which is associated with a beneficial prognosis in many tumor types. We found that cancer cell-intrinsic expression of the cytoplasmic DNA sensor cGAS is an important determinator of tumor immunogenicity.

Sensing of cytoplasmic DNA by cGAS results in production of the dinucleotide cGAMP and consecutive activation of STING followed by production of type I interferon (IFN). Type I IFNs are essential for the priming of tumor-specific effector T-cells that can attack cancer cells. Although cancer cells contain supranormal concentrations of cytoplasmic DNA, they rarely produce type I IFN spontaneously. This suggests that defects in the cGAS/STING pathway are common and may serve as immune escape mechanism.

We found that cancer cells produce cGAMP that is transferred via gap junctions to tumor-associated dendritic cells, which respond by producing type I IFN in situ. Cancer cell-intrinsic expression of cGAS – but not STING – promotes infiltration by effector T-cells and consequently, results in prolonged survival of tumor-bearing mice. Furthermore, cGAS-expressing cancers respond better to genotoxic treatments. In human colorectal adenocarcinomas, we found a correlation between T-cell infiltration and tumor-intrinsic cGAS expression, underscoring the relevance of our findings for human cancer.

Thus, cancer cell-derived cGAMP is crucial to protective anti-tumor T-cell immunity. Consequently, cancer cell-intrinsic expression of cGAS determines tumor immunogenicity. These findings are relevant for genotoxic as well as immune therapy for cancer.

101

Harnessing a bacterial-derived adhesin as novel probe to read out extracellular matrix fiber tension in tumor tissues

C. M. Fonta (1), S. Arnoldini (1), A. Moscaroli (2), M. Béhé (2), V. Vogel (1)

(1) Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, Swiss Federal Institute of Technology Zurich, Switzerland, (2) Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland

The extracellular matrix (ECM) undergoes major structural and biochemical transformations in tumorigenic microenvironments that importantly alter its composition as well as the inherent tensional state of its protein fibers. The literature broadly reports stiffer ECM fibers and altered physical features in tumor stroma, however, the lack of a probe to visualize the tensional states of ECM fibers limits the understanding of the underlying principles how the cell-ECM crosstalk is altered by tensional state alterations in the ECM. Recently,

our lab has identified a bacterial derived adhesin from S.aureus that binds with nM affinity to fibronectin fibers that are in a relaxed tensional state [1, 2]. We demonstrated the specific binding of this mechanosensitive adhesin to tumor stroma both ex vivo on mouse tumor xenograft cryosections and in vivo in tumor xenograft-bearing mice injected with the radiolabelled adhesin and imaged with SPECT/CT [3]. These results suggest the accumulation of relaxed fibronectin fibers in tumor stroma, which is counterintuitive, as the literature describes tumor microenvironments to be stiffer and denser than normal ECM. However, previous work from our lab demonstrated the accumulation of relaxed fibronectin fibers in area of enhanced collagen I fiber deposition in vitro [4]. Since nothing is known about the tensional states of ECM fibers in healthy or diseased tissues, we are performing proximity analyses asking what kind of ECM components, biomarkers and cell types are found in close proximity to relaxed fibronectin fibers in different mouse and human tumor and healthy tissue cryosections using immunohistochemistry. In summary, this bacterial adhesion is the first mechanosensitive nanoprobe that can read out the tensional state of fibronectin fibers at the tissue level. We are now exploring how fiber tension relates to tissue function and are making efforts to translate it for theranostic applications into the Clinique.

1. Chabria, M., Hertig, S., Smith, M. L. & Vogel, V. Stretching fibronectin fibres disrupts binding of bacterial adhesins by physically destroying an epitope. Nat. Commun. 1,135 (2010).

2. Hertig, S., Chabria, M. & Vogel, V. Engineering mechanosensitive multivalent receptor-ligand interactions: Why the nanolinker regions of bacterial adhesins matter. Nano Lett. 12,5162–5168 (2012).

3. Arnoldini, S., Moscaroli A., Chabria M., Hilbert M., Hertig S., Schibli R., Béhé M. & Vogel V. Novel peptide probes to assess the tensional state of fibronectin fibers in cancer. Nat. Commun. 8,1793 (2017).

4. Kubow, K. E., Vukmirovic R., Zhe L., Klotzsch E., Smith M. L., Gourdon D., Luna S & Vogel V. Mechanical forces regulate the interactions of fibronectin and collagen I in extracellular matrix. Nat. Commun. 6,8026 (2015).

102

Elucidating the mechanisms underlying radiotherapy-induced T cell-mediated anti-tumor immunity

N. I. Kapetanakis, N. Schweiger, N. Núñez, M. Van den Broek

Institute of Experimental Immunology, University of Zurich, Switzerland

Radiotherapy was introduced as cancer treatment over a century ago and is used in palliative and curative regimes. Its ability to kill tumor cells via direct or indirect DNA damage is well established. However, accumulating evidence suggests that radiotherapy can induce tumor-specific immunity via multiple mechanisms. In fact, we and others showed in pre-clinical models that clinical efficacy of radiotherapy depends on $CD8^+$T-cells. Furthermore, we discovered that radiotherapy-induced maturation of dendritic cells and protective immunity depend on local production of anaphylatoxins.

It is still unclear, however, whether the clinical response to radiotherapy depends on tumor-resident or newly infiltrating $CD8^+$T-cells. Also, whether immunogenic or "hot" tumors respond better to radiotherapy has not been experimentally addressed.

To address the first question, we used Fingolimod, an immune modulator that sequesters lymphocytes in lymph nodes, to block new infiltration of T-cells in irradiated tumors. Our preliminary results showed that the clinical response to radiotherapy was unaffected by Fingolimod. This suggests a crucial role of tumor-associated T-cells in the therapeutic response. To understand how, we now aim to elucidate how exactly tumor-associated $CD8^+$T-cells respond to radiotherapy.

To address the second question, we will apply radiotherapy to pairs of tumors that are genetically modified to modulate their immunogenicity. First experiments suggest that tumors with intrinsic expression of cGAS respond better to radiotherapy.

Together, our work will improve our understanding of how radiotherapy affects local and systemic immunity. We think that this knowledge will contribute to development of novel combinations of immune- and radiotherapy.

103

Tumor evasion from NK cell surveillance in metastatic cancer

L. Ducimetière, M. Vermeer, B. Becher, S. Tugues

Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland

Immunoediting is a process in which the immune system sculpts the highly heterogeneous tumor landscape by eliminating the most immunogenic cancer cells, thereby selecting clones that will be able to evade from it. So far, evidence in support of cancer immunoediting arises mainly from studies performed in the context of primary tumors but little is known when it comes to metastasis. Metastatic cells are exposed to further immune challenge than their primary tumor counterparts as they migrate to distant organs, where additional mechanisms are expected to take place in order for them to successfully evade the immune system. In particular, the innate lymphocytes Natural Killer (NK) cells could play a key role in this process due to their capacity to control the metastatic burden. Evading NK cell immunity could thus presumably be a requirement for tumor cells to form metastases.

We used the 4T1 breast cancer model to study tumor cell recognition by NK cells in pulmonary metastasis. We found that metastatic cells arising in NK-depleted mice are functionally different from the ones growing in immune competent wild-type (WT) mice, as they could form lung metastases less efficiently upon isolation and re-injection into immune competent animals. To further understand the possible causes of this effect, we searched for phenotypical changes between tumor cells from lungs of WT and NK cell-depleted mice and observed a differential expression of various ligands reported to trigger a NK cell response. Following transcriptome analysis of these cells by Next Generation Sequencing (NGS) and epigenetic profiling by ATAC sequencing, we now aim at identifying mechanisms and pathways through which metastatic cells can escape from NK cells during metastatic disease.

104

The role of ECM composition during osteosarcoma cell invasion

G. Moschini (1), J. G. Snedeker (1), U. Blache (1), U. Silvan (2), M. Ehrbar (3)

(1) Institute for Biomechanics, ETH Zurich, Zurich, (2) Department of Orthopedics, University of Zurich, (3) Universitätsspital Zurich

The composition, architecture and mechanical properties of the extracellular matrix (ECM) are known to impact tumor invasion. To investigate the role of specific ECM components involved in cancerous invasion we have established a 3D in vitro model based on osteosarcoma spheroids embedded in a biomimetic synthetic poly-ethyleneglycol (PEG) hydrogel [1]. The absence of further ECM proteins in this fully-defined hydrogel system allows us to analyze the impact of individual ECM components on cancer cell invasion. Specifically, we investigate the role of fibronectin (FN), collagen 1 (Col1) and collagen 3 (Col3). For that, these ECM genes were knockdowned in osteosarcoma cells (MG63) using specific siRNAs and cells were allowed to aggregate in hanging drops into spheroids before they were embedded in the hydrogels. After two days gels were fixed and fluorescently stained with phalloidin and Hoechst. Quantification of the invasion was done in terms of maximal migration distance and covered invasion area. Our results reveal only small differences in the invasion potential between Col1 and Col3 deficient-cells and the control groups. In all these groups, cancer cells formed elongated outgrowths in all direction with tight cell-cell contacts. In contrast, FN-negative spheroids appeared less compact than the control group with highly irregular shapes and only very few cell-cell contacts. Although the mechanism is still unknown, we believe that tensile forces originating by osteosarcoma spheroids could be linked to the tumor's invasive dynamics. Previous studies have observed that cells migration occurs by stretching fibronectin fibers and cellular lining up in straight lines following their orientation [2]. In 3D spheroids, fibronectin fibers might then promote cellular contractility and traction force generation facilitating cells outgrowths formation. This phenomenon could also explain the spindle-shaped morphology of Col 1 and Col 3 depleted cells in contrast with the rounded and sparse cells observed in FN knock down invasion.In addition, integrin recruitment is also affected by ECM composition. Cells bind on FN through the integrins alpha-5 beta-1 and alpha-V beta-3 while collagen associates with the integrins alpha-1 beta-1 and alpha-2 beta-1[3]. Switching the attachment of cells by silencing different genes might change cell behavior and underlie the observed cells spreading differences.

1. M. Ehrbar, et al. Biomacromolecules 8.10 (2007): 3000-3007.
2. J. Foolen, et al. PloS one 11.8 (2016): e0160369.
3. Y. Zhang, et al. Matrix Biology 40 (2014): 62-72.

105

Chronic viral infections induce persistent loss of hematopoietic stem cell function through disruption of bone marrow stromal cell networks

S. Isringhausen (1), U. Suessbier (1), L. Kovtonyuk (1), N. Kräutler (2), P. Helbling (1), A. Gomariz (1), H. C. Wong (1), A. Oxenius (2), M. Manz (1), C. Nombela-Arrieta (1)

(1) Department of Experimental Hematology, University Hospital Zurich, Zurich, (2) Institute of Microbiology, ETH Zurich, Zurich

Hematopoiesis is a highly demand-adapted and tightly regulated process that is sustained by a rare population of self-renewing, multipotent hematopoietic stem and progenitor cells (HSPCs) residing in specialized microenvironments within bone marrow (BM) cavities. During steady state, distinct stages of hematopoiesis are finely regulated by cellular networks of mesenchymal, neural and vascular origin that jointly build a basic tissue infrastructure of the BM. Viral infections act as major stressors to the hematopoietic system, inducing massive and adaptive responses in cellular output. Generally, the effects of viral challenge and ensuing inflammatory responses on hematopoietic cells have been well defined. However, whether viral infections alter BM stromal scaffolds and thereby shape hematopoietic responses remains poorly defined. By combining conventional in vitro and in vivo assays with cutting-edge 3D confocal imaging, we herein investigated the structural and functional alterations imposed on the BM after chronic infection with Lymphocytic Choriomeningitis Virus (LCMV).
Our data shows that chronic LCMV infections result in a substantial alteration of the BM endothelial and mesenchymal stromal progenitor cell populations and a decrease in their capacity to produce HSPC-sustaining factors. Moreover, using deep tissue imaging we observed a profound and durable decimation in the number and density of CXCL12-abundant reticular (CAR) cells. Functionally, upon chronic LCMV infection CAR cells were less supportive for HSC quiescence. Importantly, major damage to BM stromal integrity is accompanied by a profound and sustained reduction in the number of both HSPCs as well as hematopoietic stem cells by phenotype. Competitive repopulation assays revealed a striking and persistent loss of HSC function after chronic LCMV infection. Furthermore, our results indicate that the observed alterations in the BM are mediated by virus-specific $CD8^+$ T cells and largely depend on the production of systemic interferon alpha (IFNα) after infection. Dual blockage of type I/II IFN signaling during chronic virus infection abrogated the increase of BM $CD8^+$ T lymphocytes and subsequently also abrogated the loss of CAR cells. Finally, our results show that dual blockage of type I/II IFN signaling during chronic virus infection was able to partially recover HSC functionality as evaluated by limiting dilution transplantation.
Our observations thus indicate that chronic infections result in persistent damage to HSPC function, which can be explained by an impaired regulatory function of the stromal compartment of the BM.

106

Protein clients are differentially affected by mutations in the endoplasmic reticulum chaperone calreticulin

P. Schürch (1), T. Wildschut (1), L. Malinovska (3), V. Lysenko (1), A. Lakkaraju (2), E. Milani (3), A. Aguzzi (2), B. Wollscheid (3), A. Theocharides (1)

(1) Department of Hematology and Oncology, University Hospital Zurich, Switzerland, (2) Institute of Neuropathology, University Hospital Zürich, Switzerland, (3) Institute of Molecular Systems Biology ETH Zurich, Switzerland

Background: Calreticulin (CALR) is an endoplasmatic reticulum (ER)-resident chaperone that ensures folding of glycoproteins (GPs) such as the thrombopoietin receptor (TpoR), major histocompatibility complex I (MHC-I) and myeloperoxidase (MPO). CALR is frequently mutated in patients with myeloproliferative neoplasms (MPNs). All CALR mutants share a mutant-specific C-terminus with type-1 and type-2 variants covering over 80% of the mutational spectrum of CALR (Klampfl et al., N Engl J Med, 2013). Mutant CALR activates the TpoR and consecutively JAK/STAT signaling through a pathologic protein-protein interaction (Araki et al., Blood, 2016). Furthermore, we have previously shown that MPN patients with homozygous CALR mutations develop a maturation defect in MPO (Theocharides et al., Blood, 2016). Therefore, we hypothesize that the protein interactome of CALR and the maturation of further GPs is altered in the presence of CALR mutants. Further, we hypothesized that disease-relevant GPs that are affected by the CALR mutations could be detected by their altered structure (e.g. due to misfolding) by using a proteomic-based screen.

Methods: We first aimed to determine how the maturation of known CALR GP clients is affected by CALR mutations. Using CRISPR-Cas9, we generated a CALR knockout in the HL-60 cell line that expresses abundant levels of MPO (HL-60 CALR KO). The expression of MPO and MHC-I on HL-60 CALR KO cells and patients with CALR mutations was determined by flow cytometry. To screen for structural changes of GPs in the presence of mutant CALR chaperones, patient-derived neutrophils were subjected to limited proteolysis-coupled mass spectrometry (LiP-MS, Schopper et al., Nat Protoc., 2017).

Results: The expression of MPO was significantly reduced in HL-60 CALR KO cells resembling the phenotype observed in patients with homozygous CALR mutations. In contrast, while MHC-I expression was diminished in HL-60 CALR KO cells, patients with homozygous CALR mutations showed normal MHC-I expression. Our preliminary LiP-MS screen revealed 115 GPs with an altered structure in hetero- and homozygous patients. Most GPs that underwent structural changes in heterozygous patients were also identified in homozygous patients. Roughly, two thirds of GPs only changed structure in context of a homozygous CALR mutation but not a heterozygous mutation.

Discussion/Outlook: Together the findings from our biased approach with MPO and MHC-I suggest that CALR mutations affect the maturation of CALR protein clients in a protein-specific manner. We are currently reconstituting HL-60 CALR KO cells with either wildtype or mutant CALR proteins (type-1 or type-2) to understand how MPO and MHC-I expression are affected by the CALR mutants. The conducted pilot study shows that LiP-MS can be used to detect structural changes in the proteomes of CALR-mutated patients. To account for patient-to-patient variability we will measure more samples to identify any disease-relevant GPs affected by mutated CALR. Thus, we will conduct a more comprehensive screen of structural changes of proteins in the proteomes of MPN patients carrying either a type-1 CALR mutation or type-2 CALR mutation.

107

CALR mutations in myeloproliferative neoplasms induce adaptation and resistance to ER stress

M. H. E. Wildschut (1), M. Van Oostrum (2), P. Schürch (1), T. Wilhelm (3), M. Huber (3), B. Wollscheid (2), A. Theocharides (1)

(1) Experimental Hematology Division, University Hospital Zurich, (2) Institute of Molecular Systems Biology, ETH Zurich, (3) Institute of Biochemistry and Molecular Immunology, University Clinic Aachen, RWTH Aachen University, Germany

Introduction: Myeloproliferative neoplasms (MPN) are a family of hematopoietic stem cell disorders characterized by a high prevalence of disease-driving mutations in JAK2, MPL, and Calreticulin (CALR). Whereas JAK2 and MPL represent the family of kinases and tyrosine kinase receptors to which many oncogenes belong, CALR is best known as an endoplasmic reticulum (ER)-resident chaperone involved in folding of glycoproteins (GPs). Mutations result in formation of a novel CALR protein C-terminus and introduction of CALR mutations in mouse bone marrow leads to MPN development. CALR mutations can result in deficiency of the GP myeloperoxidase (MPO) and activate JAK/STAT signaling. To further explore the loss and gain of function effects of CALR mutations using an integrative unbiased approach, CALR mutant and knockout cell lines were compared by various technologies including proteome analyses.

Methods: CMK and K562 cells were engineered by CRISPR/Cas9 to generate CALR mutant and knockout cell lines. Proteotype analyses were performed by DIA and TMT workflows. Cell lines were exposed to various ER stressors, ER chaperone inhibitors, and UPR inhibitors. Unfolded protein response (UPR) was determined by Western Blot and qPCR of genes previously described to be involved in UPR.

Results: Proteomes of mutant and knockout cell lines showed significantly altered proteostasis compared to their respective parental wildtypes. The most significantly upregulated biological process in both CALR mutant and knockout cells was response to ER stress, showing upregulation of many key proteins including BiP/HSPA5. This upregulation is partially dependent on ATF6 activity and could be negotiated by ATF6 inhibition. Functional experiments validated upregulation of UPR proteins and higher resistance of cell lines to increases in ER stress and ER chaperone inhibition.

Conclusions: By an unbiased proteomics approach, we show that CALR mutations cause ER stress, presumably due to loss of CALR chaperone function. Upregulation of processes involved in ER stress response could increase resistance to apoptosis and present potential therapeutic targets for improving treatment of MPN patients carrying CALR mutations.

108

Resistance mechanisms in non-small cell lung cancer patients upon anti-PD-L1 therapy

S. Hiltbrunner (1), S. N. Freiberger (4), S. Kreutzer (3), N. Toussaint (2), L. Grob (2), A. Soltermann (4), A. Curioni-Fontecedro (1)

(1) Department of Hematology and Oncology, University Hospital of Zurich, Switzerland, (2) NEXUS Personalized Health Technologies, ETH Zurich, (3) Functional Genomics Center Zurich (FGCZ), University of Zurich, ETH Zurich, Switzerland, (4) Department of Pathology, University Hospital of Zurich, Switzerland

Lung cancer is a leading cause of cancer-related mortality and has a poor prognosis with a median overall survival of 10 to 12 months. The treatment of lung cancer has changed entirely over the last years, from cytotoxic therapies to more personalized treatments according the genetic profile and PD-L1 expression in the tumor.
Co-inhibitory molecules including CTLA-4 and PD-1 prevent the immune response from overshooting during infections. PD-1 and CTLA-4 are therefore often referred to as immune checkpoints. In the context of cancer, however, such molecules prevent protective effector responses. The development and clinical success of antibodies targeting CTLA-4, PD-1 or its ligands have revolutionized cancer treatment. Whereas chemotherapy and targeted therapies with small molecules act on the cancer itself, the immune checkpoint inhibitors (ICI) are releasing the break from the immune system, which leads to eradication of the tumor by the patient's own immune system. To date these treatments have become standard of care in non-small cell lung cancer (NSCLC). Many patients with NSCLC do respond initially to ICI, however, some patients develop resistance after initial responses. Mechanisms related to resistance to immunotherapy have been described for melanoma, involving the interferon-gamma pathway. Concerning thoracic malignancies, the upregulation of another immune checkpoint molecule TIM-3 was postulated in mice. However, the knowledge about such events for thoracic malignancies is still lacking.
The aim of our study is to investigate resistance mechanisms in NSCLC patients who initially responded to ICI. We have performed whole exome, whole genome and RNA sequencing on tumors from NSCLC patients before start of ICI therapy and at the time of resistance. We analysed the differences in single nucleotide variants and gene expression changes at response and at resistance. Our results suggest mutations in specific immune pathways, mainly T cell signaling pathways, in the tumors at the time of resistance. The impaired T cell signaling could lead to an impaired anti-tumor immune response and therefore to a reduced tumor control which leads to progression of the disease. Interestingly, this correlates with the upregulation of TIM-3, another immune checkpoint molecule, currently under evaluation for targeted therapies, in the tumors at the time of resistance.
Finding biomarkers of response for patients' selection is of great importance to refine current cancer treatments. Thus, investigating resistance mechanisms in order to adapt the current therapies will change treatment regimes of patients completely.

109

Assessing cancer heterogeneity among cancer types

P. Carrio Cordo, M. Baudis

University of Zurich, Institute of Molecular Life Sciences, Zurich

Despite great advances in the resolution and characterization of genomic cancer samples, the high levels of heterogeneity at multiple scales remains to be one of the main challenges in human cancer research. Copy number aberrations (CNA) are present in the vast majority of cancers, playing an important role in tumor development. Through clonal expansion of dominant subclones distinct histopathological groups will have large differences in the CNA events, affecting then their level of heterogeneity.
The aim of our study is to quantify cancer heterogeneity among cancer entities through a refined measure of dissimilarity based on CNA events among cancer entities. The use of a large data set of cancer samples will allow for a more realistic exploration of tumor heterogeneity. The results will help point specific biological mechanisms, levels of cancer subtypes across entities, which potentially could help in the improvement of patient stratification and enhancement of current cancer classifications systems.
Initial exploration of data characteristics and use of supervised methodology was used to support the selection of sparsity and data aggregation scale through Support Vector Machine (SVM) and

Generalized Linear Models (GLM). The results highlighted the power of CNA data to identify cancer type in binary classification, although with diverse classification performance for many pairs, indicating varying heterogeneity. This motivates exploration of subclasses within cancer types. Following steps include use of unsupervised methods (agglomerative, divisive, partition, and mixture) to perform cancer type sub-clustering using insights extracted from supervised methods currently applied (e.g., for defining similarity metrics). Finally assessment of heterogeneity at the effective cluster level can be done.

110

Discovery of long non-coding RNAs promoting cisplatin resistance in non-small cell lung cancer using genome engineering

R. Esposito, T. Polidori, C. Pulido-Quetglas, J. Bergadà, R. Johnson

Department for BioMedical Research, University of Bern, Bern, Switzerland

Recently, increasing evidences suggested that long noncoding RNAs (lncRNAs), a promising yet largely unexplored class of non-coding genes, play a critical role in various biological processes of tumours, and have been implicated in resistance to various drugs. Although critical roles in normal biology and disease have been revealed for a subset of lncRNAs, the function of the vast majority remains untested.

Our model of study is the non-small cell lung cancer (NSCLC), that kills around one million people worldwide every year and survival rates have improved little over the past two decades. Chemotherapy based on drugs such as cisplatin remains the standard of care for the majority of patients, but resistant clones usually occur, leading to relapse. This highlights the need to look beyond the classical proteins and pathways to identify novel therapeutic targets. Our core hypothesis is that amongst the thousands of uncharacterised lncRNAs, there exist some that play roles in chemotherapeutics response, and may be exploited as targets.

To this aim, we developed CRISPR-Cas9 genome-engineering screening to identify noncoding loci that influence the cisplatin response. High-throughput screening of lncRNAs using this method is challenging. Indels caused by a single cut in non-coding regions are unlikely to produce a functional knock-out. To address these challenges, we adapted CRISPR-Cas9 method using the validated approach of promoter deletion by a pair of gRNAs (pgRNAs) targeting the Transcriptional Start Sites (TSSs) flanking regions.

We set up a home-made bioinformatic pipeline to identify known and new lncRNAs that are active in NSCLC, and do not overlap protein-coding genes. About 1000 lncRNAs selected candidate underwent two different cisplatin-based assays: "Viability screen" to identify pgRNAs that dropped-out in living cells after cisplatin treatment; and "Death screen" to identify enriched pgRNAs in death cells. We identified 12 lncRNA loci that, upon recruitment of pgRNAs, mediate resistance to cisplatin in human NSCLC cells. Preliminary in vitro validation of 3 selected candidates confirmed the CRISPR-screen results for 2 of them, showing an increased cell sensitivity to cisplatin when knocked-out. Our screening and characterization approach provides a CRISPR toolkit to comprehensively discover new functional lncRNA loci in cancer and elucidate their diverse involvement in cisplatin response.

111

Ubiquitin phosphorylation as a new modulator of the DNA damage response

F. Walser (1), T. Gubser (1), F. Schmid (1), A. Villa (2), D. Neri (2), M. Altmeyer (4), G. Mer (5), M. Mulder (3), H. Ovaa (3), L. Penengo (1)

(1) Institute of Molecular Cancer Research, University of Zurich, Zurich, (2) ETHZ, Philochem AG, Zurich, (3) Leiden Institute of Chemistry, (4) Department of Molecular Mechanisms of Disease, University of Zurich, (5) Mayo Clinic, U.S.

Maintaining genome stability is crucial for all living cells and organisms. The repair of the most cytotoxic DNA lesions, namely DNA double-strand breaks (DSB), is based on a variety of factors that modify chromatin structure, such as kinases and ubiquitin ligases. A key event is represented by the non-canonical ubiquitination of histone H2A mediated by the ubiquitin ligase RNF168. Ubiquitinated H2A acts as a docking site for the recruitment of the downstream effectors 53BP1 and BRCA1, which promote DNA repair by either non-homologous end joining (NHEJ) or homologous recombination (HR), respectively. Although the RNF168 pathway is functionally implicated in determining the repair pathway choice, the mechanism underlying this fine regulation is still missing. Another level of complexity has been recently added to the ubiquitin code by the discovery that ubiquitin itself undergoes PTMs such as acetylation and phosphorylation.

Our research aims at characterizing the potential role of ubiquitin phosphorylation in the context of the DNA damage response (DDR). Interestingly, our data indicate that ubiquitin phosphorylation modulates the dynamics of chromatin ubiquitination and DDR activation.

Furthermore, we found that ubiquitin phosphorylated at a specific site impairs 53BP1 foci accumulation upon genotoxic stress, suggesting that phosphorylation on this site interferes with 53BP1 recruitment to damaged chromatin. By using a phospho-ubiquitin specific antibody, we found a role of this modification in finely tuning the accumulation of 53BP1 to chromatin structure. Future studies are needed to further investigate the mechanism of ubiquitin phosphorylation in the regulation of genome stability by controlling 53BP1 accumulation at chromatin structures. A better understanding of the molecular pathways regulating DNA repair will be crucial to establish novel therapeutic strategies to prevent genome instability and cancer formation.

112

Exploring genetic landscape in cancer - varied occurrence and outcome among populations

Q. Huang, M. Baudis

University of Zurich, Institute of Molecular Life Sciences, Zurich

As life expectancy increases in modern days, cancer as an aging-related disease strikes in larger scope and progressively burdens the health care system. Statistics show that the incidence and severity vary between individuals with different genetic background. Scientists world-wide are struggling to bring forward better understanding to improve treatment. However, the impact of these findings can be limited because the patients recruited for the study often originate from similar geographical location and therefore have similar background. This renders them incomprehensive representation of the disease and the research outcome may be a special case of all possible disease mechanisms.
Our research group develops and maintains the arrayMap database, which curates all the publicly available cancer genome profiling data, thus providing us a unique position for this type of study. In this project, we have integrated all the cancer studies by type and stratified the population-specific disease-associated genetic elements for the disease - 29 different cancer types, each with at least 80 samples per studied population group. Concretely, thanks to the population stratification tool recently developed in our group, we first assembled patients of similar genetic background. Then we estimated the linked genomic regions and calculated the correlation between their status and the phenotypes extracted from cancer samples. With this approach, we are proposing candidate genomic regions, with some of them having confirmed implications in cancer development. These results will help to develop group-specific drug application, create a better model for cancer prediction and further improve the understanding of the mechanism of action.

113

Telmisartan sensitizes A375 cells to BRAF targeted therapy in vitro

M. Pavlovic, J. Grahovac, T. Srdic-Rajic, S. Radulovic

Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade

Introduction of immunotherapy and mutation-based targeted therapy significantly increased response rates and overall survival rates in patients suffering from aggressive malignancies, such as melanoma. Nearly 50% of melanoma tumors carry BRAF V600E mutation, but despite the effective initial response to BRAF inhibitors, development of treatment resistance invariably occurs. BRAF inhibitors hamper glycolysis, but increased autophagy and oxidative phosphorylation limit their efficacy. Antihypertensive telmisartan is the angiotensin receptor 1 (AGTR1) antagonist and a partial agonist of the peroxisome proliferator-activated nuclear receptor γ (PPARγ) with reported anticancer properties. We examined the in vitro effects of telmisartan in a panel of human melanoma cell lines and normal dermal fibroblasts, and compared it to the pure AGTR1 antagonist losartan and the pure PPARγ agonist pioglitazone. While losartan had no effects on cell viability, telmisartan was as effective, or better than pioglitazone in reducing melanoma cell viability. At concentrations achievable in patients, telmisartan had no significant effects on fibroblast viability, while BRAF mutant melanoma cell lines were especially sensitive. Telmisartan induced fragmentation of mitochondria, release of reactive oxygen species and ultimately apoptosis in BRAF mutant and BRAF wt melanoma cells. As mitochondrial fusion and switching to OXPHOS is one of the mechanisms of resistance to BRAF inhibitors, we hypothesized that telmisartan by disruption of mitochondria may sensitize melanoma cells to targeted therapy. Indeed, in combination with BRAF inhibitor vemurafenib in A375 cells low dose telmisartan had synergistic effects. More importantly, telmisartan treatment was effective in vemurafenib-resistant A375 cell line and improved its response to vemurafenib. Our findings provide a scientific rationale for a retrospective study in patients of whether telmisartan influences progression and therapy outcome in BRAF mutant melanoma.

114

LIGHT/LTβR signaling regulates self-renewal and differentiation of hematopoietic and leukemia stem cells

S. Hoepner (1), R. Radpour (2), M. A. Amrein (2), D. Koller (2), G. M. Baerlocher (3), C. Riether (1), A. F. Ochsenbein (1)

(1) Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, Switzerland, (2) Department for BioMedical Research, University of Bern, Bern, Switzerland, (3) Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital, University of Bern, Switzerland

Aim: Hematopoietic stem cells (HSC) are responsible to replenish all blood cell lineages. The balance between self-renewal, proliferation and quiescence is tightly regulated to ensure the maintenance of the stem cell pool but also guarantee rapid adaptation in response to hematopoietic stress. Likewise, the balance of self-renewal and differentiation is critical in the pathology of hematopoietic malignancies. Here we show that lymphotoxin-beta receptor (LTβR), a member of the TNF superfamily, and its ligand TNFSF14 (LIGHT) play an important role in HSC and leukemic stem cell (LSC) regulation. LIGHT/LTβR signaling maintains stem cell quiescence, promotes symmetric division and thereby contributes to HSC and LSC self-renewal.

Material and Methods: To study LIGHT/LTβR signaling under hematopoietic stress, we administered 5-Fluoracil (5-FU) a genotoxic stress agent into Ltbr KO, Light KO and C57/BL6 (WT) mice or performed serial competitive repopulation assays. Cell cycle activity, cell viability and cell division of HSCs were analyzed. In a second approach we investigated LIGHT/LTbR signaling in LSCs, using a CML mouse model. The relevance of this signaling pathway was further validated in human stem- and progenitor cells (HSPCs) and LSPCs from G-CSF mobilized patients and CML patients, respectively.

Results: We show that Ltbr- and Light-deficiency on murine HSCs and LSCs resulted in enhanced proliferation, asymmetric division and a reduced stem cell pool. LTβR signaling was induced by LIGHT in an autocrine manner. Moreover, LTBR-, and LIGHT-knockdown on human HSPCs reduced their capacity to form colonies in vitro. Similarly, LTBR-knockdown on human LSPCs resulted in decreased colony formation in vitro, indicating that the LTβR pathway affects the stemness of human activated HSCs and LSC.

Conclusion: Thus, our results demonstrate, that LIGHT/LTβR signaling is a novel player in murine and human HSC and LSC self-renewal, which potentially provide a new strategy to eliminate LSCs.

115

Identification of proteins associated with RNA:DNA hybrids formed upon replication stress

B. Boleslavska (1), J. Dobrovolna (2), Z. Nascakova (2), A. Oravetzova (2), P. Janscak (3)

(1) Department of Cellular and Developmental Biology, Faculty of Science, Charles University, Prague, (2) Institute of Molecular Genetics, AS CR, Prague, (3) Institute of Molecular Cancer Research, University of Zurich

Maintaining the genome integrity is one of the fundamental features of life. DNA damage can cause cell death or chromosomal rearrangements, which can promote cancerous growth. One of important sources of DNA damage are R-loops, three-stranded structures, which arise when nascent RNA strand is annealed to the DNA template strand. Unpaired complementary DNA strand is then vulnerable to breakage and nucleotide modifications. In organism, R-loops may arise due to DNA replication stress, but also have physiological functions. However, more detailed information about the metabolism of R-loops is not available yet.

We established a stable cell line inducibly expressing a catalytically-inactive form of RNaseH1 (D210N) fused with a promiscuous biotin ligase, termed BioID2 (Biotin IDentification2), and an HA epitope tag. This RNH1(D210N)-BioID2-HA chimeric protein is targeted to R-loops through RNH1(D210N) binding to RNA:DNA hybrids and biotinylates neighboring proteins upon addition of biotin to the cell culture medium. These biotinylated proteins can be then isolated with streptavidin-coated beads and identified using mass spectrometry. Our goal is to identify the proteins associated with R-loops formed under conditions of replication stress and to determine their roles in the maintenance of genomic stability.

116

The role of NK cells in development and progression of liver metastasis

G. Lucchiari* (1), L. Ducimetière* (1), D. Burri (2), L. Heeb (1), J. Gschwend (1), N. Nunes (1), V. Cecconi (1), B. Burkhard (1), S. Tugues Solsona# (1), M. Van den Broek# (1)
* shared first author contribution; # shared senior authorship

(1) Institute of Experimental Immunology, University of Zurich, Zurich, (2) Department of Biosystems Science and Engineering, ETH Zurich Basel

Natural killer (NK) cells are important immune effector cells, which bridge innate and acquired immunity in controlling tumors and viruses. Most of our current knowledge of NK cells is derived from studies of mouse splenic and human peripheral blood NK cells, referred to as conventional NK cells. Recently, a population of tissue-resident NK cells was described in the human and mouse liver. In the liver, tissue-resident NK cells differ from conventional NK cells in terms of surface marker expression, cytokine profiles and cytotoxic potential. In the context of cancer, conventional NK cells are particularly important in protection against metastatic seeding to the lungs, suggesting that they target circulating tumor cells. The effect of tissue-resident NK cells on liver metastasis is largely unknown. We therefore aimed to investigate the effect of tissue-resident and conventional NK cells on liver metastasis.
We used two different cell lines, MC38 and LLC, to induce liver metastasis in syngeneic C57BL/6 mice. Using high-dimensional flow-cytometry analysis, we observed that metastatic nodules differently affect tissue-resident and conventional NK cells. Whereas conventional NK cells gradually lost their function with progressing metastasis, tissue-resident NK cells remained fully functional.
We used different genetically modified mouse strains that lacked the expression of the transcription factor Nfil3 or Hobit to study the individual role of conventional or tissue-resident NK cells in liver metastasis, respectively. We found that absence of either NK cell population during the entire metastatic process resulted in increased metastatic load.
Taken together, we think that conventional NK cells are important during metastatic seeding, whereas tissue-resident NK cells restrict metastatic outgrowth. Our findings suggest that stimulating tissue-resident NK cells is a promising approach to treat established liver metastasis.

117

A deep learning pipeline for spatial characterization of bone marrow stroma

A. Gomariz (1), S. Isringhausen (1), P. M. Helbling (1), U. Suessbier (1), O. Goksel (2), M. G. Manz (1), C. Nombela-Arrieta (1)

(1) Hematology, University Hospital and University of Zurich, Switzerland, (2) Computer Vision Laboratory, ETH Zurich, Switzerland

Bone marrow (BM) cavities are the primary sites of blood cell production, which is sustained by a rare population of self-renewing and multipotent hematopoietic stem cells (HSC). Local cues deriving from non-hematopoietic BM stromal cells of vascular, mesenchymal or neural origin critically modulate hematopoiesis and HSC maintenance through cell-cell interactions. Among stromal components, perivascular mesenchymal CXCL12-abundant reticular cells (CARc) and endothelial cells lining sinusoidal BM microvessels (sinusoids) have been shown to fulfill prime roles in the orchestration of hematopoietic development. Thus, the study of spatial distributions of different BM components can reveal key information on cellular crosstalk and the molecular mechanisms underlying hematopoietic regulation.
Understanding how cells interact with their microenvironment requires imaging the tridimensional spatial context surrounding them. For this, we have established advanced tissue processing and clearing protocols for the generation of 3D microscopy reconstructions of entire BM cavities with subcellular detail. To generate a high-throughput and unbiased analysis, we have developed a deep learning approach for automatic detection of the observed cellular components, which are then represented as segmented objects. We subsequently used robust spatial statistics to quantify how these segmented structures mutually constrain the available volume and interact with each other within the tissue boundaries.
Applied to our BM datasets, these methods are used to segment 3D sinusoidal microvascular networks with unprecedented speed and accuracy. The sinusoids are seen to occupy 20% of the total BM volume and leave little space for other cellular populations. We use classical segmentation methods to automatically detect the positions of CARc and to report their preferential location in perisinusoidal regions, with 64% of them being in direct contact with the abluminal side of endothelial cell walls.
In the BM, the results suggest that the stromal components have been previously inaccurately characterized, and we have proposed rigorous descriptors of their spatial confinement and cell frequencies.

Furthermore, this approach can be used for uncovering novel spatial phenotypes of immunostained cellular components in different organs and conditions, including tumorous tissues.

118

Role of CXCL13 and LTαβ in development of tumour-associated tertiary lymphoid structures

F. Movahedian Attar, K. Silina, M. Van den Broek

Institute of Experimental Immunology, University of Zurich

Tertiary lymphoid structures (TLS) resemble follicles of secondary lymphoid organs (SLOs) and can occur in tumours. High TLS density correlates with better prognosis of cancer patients, but it is not known why some patients develop more TLS than others. Both CXCL13 and lymphotoxin-alpha beta (LTαβ), that have established roles in SLO formation, are also expressed in various human cancer tissues including lung and breast carcinomas. Therefore, we aimed to investigate the roles of these molecules in development of tumour-associated TLS in murine models.

We found that alum/OVA-induced TLS develop through three consecutive steps: (i) B cell aggregates (early TLS), (ii) B and follicular dendritic cells (primary follicle-like; PFL), and (iii) germinal centre-positive TLS (secondary follicle-like; SFL). Initiation and maturation of TLS happened independent of IL-33 signalling in this experimental system.

Furthermore, we overproduced CXCL13 and LTαβ in Lewis lung carcinoma (LLC), $Kras^{G12D}$;$P53^{-/-}$ lung adenocarcinoma (KPL), and breast carcinoma (4T07) cell lines and injected the cells intravenously to model lung cancer. Overexpression of these factors initiated the first step of TLS development in case of 4T07 and KPL but not of LLC tumours. In addition, TLS density inversely correlated to the tumour mass in 4T07 tumours (Pearson`s r=-0.83, p=0.02).

Our results suggest two important concepts. First, some tumours (e.g. LLC) intrinsically hamper development of TLS. Second, a high density of TLS and high tumour load seem mutually exclusive, suggesting a causal relationship. Using these novel models, we will study factors that prevent or support the development of cancer-associated TLS.

119

Tumor suppressive mechanisms of Gastrokine 1 in pancreatic carcinogenesis

S. Steiner (1), T. Reding (1), D. Lenggenhager (2), M. E. Healy (2), A. Gupta (1), G. Wanner-Seleznik (1), R. Graf (1)

(1) Department of Visceral and Transplantation Surgery, University Hospital Zurich, Zurich, (2) Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich

Introduction: Late detection of pancreatic ductal adenocarcinoma (PDAC) and limited treatment options lead to poor survival of PDAC patients. A better understanding of the pathomechanism leading to PDAC may help to identify biomarkers for early detection. Pre-malignant lesions such as pancreatic intraepithelial neoplasia (PanIN) follow a multistage development process to form PDAC. While studying a K-Ras driven mouse model (KC mice) of PDAC, which recapitulates the stepwise progression of pancreatic cancer, we serendipitously identified Gastrokine 1 (GKN1) in early PanIN lesions. GKN1 is a stomach-derived secreted protein that maintains gastric homeostasis. Loss of GKN1 in gastric cancer suggested that it might act as tumor suppressor. We further confirmed presence of GKN1 in human PDAC samples, and its secretion in cystic lesions. The discovery of GKN1 in pancreatic carcinogenesis is of great importance, as normal pancreas do not express GKN1.Therefore, we aim to investigate the function of GKN1 in the pancreas, to understand the early events that underlie the development of premalignant lesions leading to pancreatic carcinogenesis.

Methods: We intercrossed KC mice with GKN1-/- mice. GKN1-/- KC & GKN1+/+ KC pups were analyzed at the age of 3- and 9 months for the quantification of PanIN lesions and appearance of tumors via histology. Furthermore, mRNA transcripts were also analyzed to assess genes (relevant for apoptosis, EMT or tissue remodeling and stroma) involved in tumorigenic processes. Using IHC, pancreatic tissues were analyzed to quantify tumor stroma, markers of cell proliferation and apoptosis.

Results: As noticed previously, GKN1 expression is restricted to low grade premalignant lesions. Analysis of pancreatic tissue from 3 months old GKN1-/- KC mice, showed more PanINs compared to GKN1+/+ KC mice. A significant difference in the mRNA screen revealed a different apoptosis regulation in GKN1-/- KC mice. IHC analysis of Cleaved caspase-3 also suggested decreased apoptosis in the

absence of GKN1. We also noticed a significant decrease in Fas protein, a marker for extrinsic apoptosis pathway, in these animals. Nine months old GKN1-/- KC mice, showed increased tumor incidence (30% vs 12.5%). The histological comparison at 9-months showed that GKN1-/- KC mice developed a collagen-rich dense stroma around the pancreatic lesions compared to sparse stroma in KC animals.

Conclusions: We conclude that the absence of GKN1 leads to accelerated PanIN development. We suggest that GKN1 influences apoptosis avoidance at an early age and development of dense stromal reaction in later stages leading to an increased tumor incidence. In summary, we confirm that GKN1 acts as tumor suppressor in pancreatic cancer.

120

Notch signaling inhibition in glioma cells alters the tumor microenvironment and disease progression

E. Parmigiani (1), C. Rolando (1), R. Ivanek (1), K. Eschbach (2), C. Beisel (2), R. Tussiwand (1), V. Taylor (1), C. Giachino (1)

(1) Department of Biomedicine University of Basel, Switzerland, (2) Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

Notch signaling is believed to be oncogenic in glioma, primarily by virtue of its stem cell promoting activity. However, surprisingly, inactivating mutations in Notch pathway components have been identified in glioma subtypes and Notch inhibition dramatically accelerates tumor progression in mouse models of glioma subtypes. In order to investigate the mechanisms underlying the tumor suppressive function of Notch in neural stem and progenitor cells, we combined conditional genetics in mouse models with expression profiles analyses of Notch-signaling-depleted tumors. We found that Notch inhibition in tumor cells downregulates expression of genes associated with quiescence of neural stem cells and releases expression of genes involved in stem cell activation and cell cycle progression, thereby promoting an active proliferative state. Unexpectedly, blocking Notch also downregulates genes associated with recruitment and activation of immune cells. This results in an impaired microglia activation at early stages of tumor development that hampers a proper immune response. Interestingly, individual Notch receptors have distinct functions during glioma development, and only specific Notch receptors or receptor combinations can activate a tumor suppressive signal. Our data indicate that Notch suppresses glioma formation by regulating both intrinsic and extrinsic properties of tumor cells, and that distinct Notch receptor paralogues are differently engaged in glioma progression.

121

In silico analysis of nischarin expression in pancreatic ductal adenocarcinoma reveals its potential tumor-suppressive role

M. Vidosavljevic, S. Radulovic, J. Grahovac

Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies. Despite significant effort in developing novel detection and therapeutic strategies, the five-year survival rate remains just around 5–7% and has not changed in the last 40 years. Therefore, novel targets and agents that target PDAC are of great interest. Nischarin (NISCH, IR1, IRAS) is a non-adrenergic imidazoline-1 receptor protein that localizes to the cytosol and binds the cytoplasmic tail of integrin α5. It directly interacts with PAK1, LIMK, Rac1, and LKB1 and inhibits cellular migration and invasion of epithelial cells, and has been described as a tumor suppressor in breast and ovarian cancer. NISCH expression and its biological role in other types of cancer have not been investigated to date. The aim of this study was to examine the expression of nischarin in PDAC and its potential role in the disease progression. The interactive web resource for analyzing cancer transcriptome data from TCGA database – UALCAN – was used to analyze the NISCH mRNA expression levels in normal pancreas and PDAC samples. A declining trend was observed in PDAC tumor tissues compared to the normal. To assess the effects of nischarin on patient survival we divided patients from the TCGA PDAC database (n=174) into two groups based on the mean of NISCH expression in the entire population. Kaplan-Meier survival analysis showed that patients with lower NISCH levels had statistically significant decrease in overall survival compared to the patients with higher NISCH expression (log-rank P = 0.0277, hazard ratio High/Low=0.59). We confirmed these findings in an independent GEO dataset (GSE28735), where higher NISCH mRNA expression in PDAC patients correlated with better prognosis (log-rank P=0.0247, hazard ratio High/Low=0.47). To get better insight into the possible role of NISCH we performed Gene Set Enrichment Analysis (GSEA) of several publicly available datasets. Samples were divided into $NISCH^{high}$ and $NISCH^{low}$ group by selecting

the top and bottom 25% NISCH expressing tumors, and analyzed with the GSEA Broad Institute software using the Hallmark gene sets. We found that in NISCH^{low} group gene sets previously reported to be involved in poor therapy response and worse outcome were enriched: regulation of cell cycle, G2/M checkpoint, unfolded protein response, MYC targets and mTOR signaling. These findings warrant further investigation into the functional role of nischarin and whether it can be a prognostic marker in PDAC. Given that nischarin is a druggable target, with endogenous ligand agmatine and chemical ligand moxonidine, it is potentially a new therapeutic target for more effective PDAC treatment.

122

A CRISPR/Cas9 domain screen identifies a small motif in the PAX3-FOXO1 transactivation domain relevant for tumor maintenance in alveolar Rhabdomyosarcoma

K. H. Holste, D. O. Laubscher, M. A. Wachtel, B. E. Schäfer

Experimentelle Infektiologie und Krebsforschung, Kinderspital Zürich

Fusion transcription factors are the main drivers of tumorigenesis in many pediatric leukemia and sarcoma. Since these tumors usually are also characterized by a quiet mutational landscape and a paucity of druggable oncogenes, the fusion proteins often remain as sole relevant drug targets. However, transcription factors are challenging targets for small molecule inhibitor development, since they lack enzymatic activities and, apart from the DNA binding domains, are usually intrinsically disordered. Identification of druggable structures in such proteins is therefore of great clinical interest.
One example for such a fusion transcription factor is PAX3-FOXO1 (P3F), the major driver and pathognomonic marker of alveolar Rhabdomyosarcoma. The oncogene results from fusion of the DNA binding domain of the paired box protein 3 (PAX3) to the transactivation domain (TAD) of FOXO1. The result is a new, functionally highly active transcription factor responsible for transformation.
Our goal here was to identify new options for direct targeting of P3F. Towards this aim, we made use of the CRISPR/Cas9 technology to perform a screen for identification of important functional domains of P3F. This screen revealed that both DNA binding domains within the PAX3 region are highly important, as expected for a transcription factor. Importantly however, the screen also identified a small domain within the TAD of the FOXO1 part, which is absolutely required for the function of the protein.
This domain is roughly 40 amino acids long and is located at the C-terminal end of the protein. By testing the effect of different mutations in this domain in reporter assays, we identified a single cysteine whose mutation leads to more than 50 percent reduction in transcriptional activity. Hence, this cysteine seems to be a key player for the transactivation capability of P3F and therefore tumor maintenance of aRMS.
These results suggest that interference with the function of this domain, either directly by targeting the cysteine or via interference with protein-protein interactions could be a promising new avenue for therapy of aRMS. Based on these results we plan to identify interacting proteins by immunoprecipitation followed by mass spectrometry and in parallel will purify this small motive to find small molecules that directly bind to this region.

123

Epigenetic reprogramming for reversal of targeted-therapy resistance and melanoma invasion

A. S. Cereghetti (2), L. P. Ferretti (1), R. Dummer (2), M. O. Hottiger (1), M. P. Levesque (2)

(1) Department of Molecular Mechanisms of Disease, University of Zurich, (2) Department of Dermatology, University Hospital Zurich

Metastatic malignant melanoma is the deadliest form of skin cancer. In case of early detection, surgical removal and lymph node biopsy yield high patient survival rates. For advanced melanoma, the gold standards of therapy are MAPK inhibitors (MAPKi) and immune checkpoint inhibitors, but median overall survival is still limited to 33.6% and 63%, respectively. On one hand, activating mutations of the MAPK pathway lead to resistance acquisition to MAPKi within 9 to 11 months. On the other hand, only a minority of patients obtains durable benefit from immune checkpoint inhibitors mostly due to a priori existing resistance driven by tumor heterogeneity, lack of tumor-infiltrating lymphocytes or induction of severe side effects. Remarkably, phenotype plasticity leading to acquisition of MAPKiresistance and metastatic behavior was linked to a process resembling the epithelial-to-mesenchymal transition (EMT), in melanoma as well as in other solid cancers. Emerging evidence suggests that epigenetic modifications promote this EMT-like melanoma phenotype switch from an epithelial (proliferative) to a mesenchymal (invasive) phenotype. Despite a growing understanding of the epigenetic modifications that regulate most EMT-related transcription factors, epigenetic mechanisms

driving melanoma plasticity and drug resistance remain elusive. Hence, understanding how epigenetic alterations contribute to drug resistance in melanoma may facilitate the development of novel anticancer therapies or drug combinations. To this purpose, we will perform a drug screening on multicellular tumor spheroids taking advantage of MAPKi-resistant patient-derived melanoma cells provided by the Biobank of the University Hospital of Zurich. The effect of selected FDA-approved epigenetic inhibitors in combination with MAPKi on the expression of more than 350 melanoma surface proteins will be quantified to understand how their expression is influenced by epigenetic regulation. This will help us elucidate new vulnerabilities that could be targeted in future therapies, for instance by identifying epigenetic drugs that increase a tumor's immunogenicity.
Second, by performing in vitro and in vivo invasion assays, we will define how epigenetic reprogramming may influence the melanoma cell's ability to switch to a mesenchymal fate and thus leading to therapeutic resistance and acquisition of a metastatic behavior. These experiments will improve our understanding of how epigenetic regulation may be used in combination with MAPKi to prevent tumor cell fate switching, immunosuppression, and the development of therapeutic resistance in the clinic.

124

Normal human immune cells and IL-6 promote growth of primary and transformed DLBCL cells in experimental orthotopic models of the disease

H. Hashwah (1), K. Bertram (1), A. Stelling (1), M. Manz (2), A. Theocharides (2), A. Tzankov (3), A. Müller (1)

(1) Institute of Molecular Cancer Research, University Zurich, Zurich, (2) Department of Hematology, Universitätsspital, Zurich, (3) Institute of Pathology, University and University Hospital Basel

An important obstacle in Diffuse Large B-cell Lymphoma (DLBCL) research is the very heterogeneous nature of the disease, which is currently not well reflected in mouse models. In an attempt to address this, we set out to establish serially transplantable, orthotopic primary DLBCL models in third generation humanized mice, more specifically MISTRG mice. MISTRG express four human cytokines (M-CSF, IL-3, Sirp1α, thrombopoietin, GM-CSF) in the $Rag2^{-/-}IL2Rg^{-/-}$ background that were knocked into the respective murine loci, and therefore are particularly permissive to engraftment of B-cells and various myeloid cell compartments.
We could additionally identify a role for hIL6 as an important factor that aid engraftment of DLBCL cell lines and primary patient material in MISTRG mice.

125

Characterization of detoxification mechanisms in pancreatic cancer

V. Thiel (1), W. Nadler (1), A. Kerner (1), B. Klaus (2), M. Reitberger (1), L. Kuhlmann (1), A. Muckenhuber (3), W. Weichert (3), O. Kossi (1), V. Vogel (1), C. Rösli (1), A. Trumpp (1), M. Sprick (1)

(1) German Cancer Research Center (DKFZ) and Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), Heidelberg, Germany, (2) European Molecular Biology Lab, Centre for Statistical Data Analysis, (3) Institute of Pathology, Technical University Munich and German Cancer Consortium (DKTK; partner site Munich), Munich, Germany

Pancreatic ductal adenocarcinoma (PDAC) accounts for > 85 % of pancreatic tumor cases and is the fourth most frequent cause of cancer death in the western world. PDAC is one of the most aggressive tumor types with extremely poor prognosis. Absence of early symptoms of pancreatic ductal adenocarcinoma (PDAC) commonly prevents timely diagnosis and current therapies only offer modest benefits to patients suffering from this fatal disease. This is largely due to the lack of effective therapies for this disease and the development of secondary drug resistance. These mechanisms of drug resistance are poorly understood. This is in part due to the extensive inter- and intra-tumor heterogeneity of this cancer and lack of adequate models that represent this complexity. Recent findings by Collisson et al. and Noll et al. suggest the existence of different molecular subtypes in PDAC and their impact on patient outcome. Proteomic profiling of PDAC subtypes, which differ in overall survival and therapeutic response [1], using a prefractionation-based label-free MALDI/MS approach enabled the identification of >5600 proteins from patient-derived in vitro PDAC models. Interaction and enrichment analysis of differentially expressed proteins revealed systematic alterations of pathways involved in xenobiotic metabolism. While the importance of CYP3A5 for drug detoxification in exocrine PDAC has recently been demonstrated [1], our data suggest that further members of this family might mediate particular drug resistance.

We now showed that several other enzymes and transcription factors play a major role in the sensitivity to and detoxification of current chemotherapeutics of the FOLFIRINOX treatment regime.
1. Noll et al., Nat. Med. 2016

126

Dasatinib interferes with lymphocyte migration in vivo
A. Marcos-Jimenez (1), C. Munoz-Calleja (2), J. V. Stein (1)

(1) Department of Oncology, Microbiology and Immunology; University of Fribourg, Fribourg, (2) Department of Immunology; La Princesa University Hospital, Madrid

Dasatinib is a dual ABL/SRC family tyrosine kinase inhibitor approved for the treatment of chronic myeloid leukemia and Philadelphia-positive acute lymphoblastic leukemia. Although very effective and well tolerated, patients usually exhibit some side effects. One of them is the presence of a transient lymphocytosis that peaks 2 hours after the oral intake of the drug and resolves spontaneously reaching basal levels around 4 hours later. The actual cause of this side effect is currently undetermined. In this study, we hypothesize that dasatinib interferes with lymphocyte trafficking. Using an in vitro chemotaxis assay we demonstrated that in the presence of dasatinib, B cells show a reduced migration towards CCL21, CXCL12 and CXCL13, while T cells remain unaffected. We confirmed that this effect on B cells is not due to a change in the expression of chemokine receptors or an increased apoptosis. In order to confirm this finding in an in vivo situation, we performed two-photon microscopy in the popliteal lymph node of mice treated with dasatinib. To our surprise, the migration of both B and T lymphocytes within the parenchyma of the lymph node was almost completely abolished. The differences in migration that T lymphocytes display when exposed to dasatinib in vivo and in vitro suggest that the effect on other cells may contribute to the phenomena of the lymphocytosis. Thus, future investigation will focus on the effect of dasatinib on stromal cells, which play an important role in the maintenance of the microenvironment in lymphoid organs.

127

The role of the Notch pathway in platinum-based therapies
S. Bernardo, H. Tourriere, E. Bousquet, A. Giry, L. Linares, C. Gongora, A. Maraver

Institut de Recherche en Cancérologie de Montpellier (IRCM), Université de Montpellier, Institut Régional du Cancer de Montpellier (ICM), Montpellier, 34298, Cedex 5, France

Lung cancer is the leading cause of cancer death among men and women worldwide. Despite the major advances in the treatments, platinum-based therapy remains the standard of care for patients affected by KRAS-driven lung cancer. Even though the platinum-compounds display an initial effect on these patients, the onset of the relapse constitutes the main challenge for the clinic.
The molecular mechanisms underlying lung adenocarcinoma (LUAD) relapse are not completely elucidated yet, thus it is fundamental to understand them in order to improve patients survival. Our data show that upon carboplatin treatment, the Notch pathway gets activated in vitro. Since this effect was also true for several other DNA damage insults, our main hypothesis connects the DNA Damage Response (DDR) to the activation of the Notch pathway. Therefore, we are working on understanding how this interplay occurs.
Importantly, our preliminary data indicates that protein kinase ataxia telangiectasia-mutated (ATM) is a key mediator in the activation of the Notch pathway during DNA damage signalling. ATM is well-known as the chief mobilizer of the cellular response to the most toxic lesions to the DNA, the double-strand breaks which are also the type of damage caused by the conventional chemotherapy. Among the several substrates of ATM in response to the damage, there is MDM2 (mouse double minute 2) that it has been shown to interact with the Notch pathway. Therefore, we have analysed for a direct effect of MDM2 in Notch activation upon DNA damage. Our data show a new axis by which ATM, MDM2 and Notch can crosstalk during DDR and provide pro-survival signalling to cancer cells.
Using LUAD Patient-Derived Xenografts we are at the moment translating our molecular mechanistic into new treatments for carboplatin-resistant LUAD cells by combining Notch and MDM2 inhibitors with carboplatin.
Our results can offer a new therapeutic window for KRAS-driven LUAD that become resistant to platinum-based therapy, hence tackling an urgent and unmet clinical need.

128

Trop2 signaling in regulation of proliferation and cell survival in low serum conditions

S. Lenárt (1), T. Kucírková (1), F. Trcka (2), J. Remšík (3), K. Soucek (3), J. Šmarda (1), P. Beneš (4)

(1) Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic, (2) Regional Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Zluty Kopec 7, 656 53 Brno, Czech Republic, (3) Institute of Biophysics of the Czech Academy of Sciences, Královopolská 135, 612 65 Brno, Czech Republic, (4) Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's University Hospital Brno, Pekarská 53, 656 91 Brno, Czech Republic

Carcinomas, the most common type of tumors, often overexpress a transmembrane glycoprotein Trop2. Expression of this protein has been shown to correlate with tumor aggressiveness, metastases, and poor prognosis. Trop2 is therefore considered as suitable antigen for targeted therapy. However, our knowledge regarding the molecular mechanisms of Trop2 function in cancer cells is incomplete. To investigate Trop2 function to cell response to stress, we overexpressed wt and mutant Trop2 proteins in MDA-MB-231 breast cancer cell line. We show that cells overexpressing wt Trop2 are more resistant to starvation which is consistent with their lower proliferation activity and activation of Akt kinase. Moreover, we found that regulated intramembrane proteolysis of Trop2 is necessary for Akt activation and both regulated intramembrane proteolysis and phosphorylation of serine 303 are necessary for activation of beta-catenin signaling pathway. Our findings provide a novel insight into Trop2 signaling and function in cancer cells with potential implications for future research and therapies.

This work was supported by Project ENOCH No. CZ.02.1.01/0.0/0.0/16_019/000086 from the European Regional Development Fund, and MUNI/A/0824/2017 from Grant Agency of the Masaryk University.

129

Crucial role of CDCP1 in prostate cancer development and progression using novel transgenic mouse model

M. D'Ambrosio, A. Alajati, A. Alimonti

IOR Institute of Oncology Research, Bellinzona

Elevated levels of CUB domain-containing protein 1 (CDCP1) have been reported to be associated with poor prognosis in several human malignancies, including prostate cancer. However, its oncogenic role remains unexploited. Taking an advantage of different mouse models, we demonstrate that CDCP1 per se is an oncogene. The overexpression of CDCP1 in prostatic epithelial cells initiates hyperplasia, which eventually develops high-grade prostatic intraepithelial neoplasia. The functional importance of CDCP1 in tumorigenesis was further fortified in Pten loss context. PTEN deficient prostate tumours fail to acquire metastatic potential in vivo because of the senescence response. CDCP1 overexpression in this context led to the development of castration-resistant prostate cancer and the dissemination of metastasis. Mechanistically, we demonstrate that overexpression of CDCP1 triggers the activation of the SRC-Myc axis and the bypass of the TGF-β-dependent checkpoint and senescence barrier driven by Pten-loss. In addition, we showed that targeting CDCP1 antagonizes c-Myc expression to block tumorigenesis by reactivating cellular senescence in human prostate cancer cells. Thus, our complementary approaches identify CDCP1 on one hand as a powerful oncogene and on the other hand as a potential therapeutic target for prostate cancer therapy. Furthermore, it could be used also as a carrier to drive the chemotherapeutic drugs directly in the tumor cells that present CDCP1 on the membrane.

130

Targeting cellular senescence

M. Colucci, A. Revandkar, A. Alimonti

IOR Institute of Oncology Research, Bellinzona

Cellular senescence is a stable cell growth arrest that occurs in tumour cells subjected to different stress including treatment with chemo-radiotherapy or targeted therapies. PTEN is one of the most frequently altered tumour suppressor genes in prostate tumours and PTEN loss can drive senescence in the "PTEN Loss Induced Cellular Senescence" (PICS). Several findings in vivo demonstrate that senescence limits tumour progression. Although arrested, senescent tumour cells remain metabolically active and secrete a variety of cytokines and inflammatory factors known as the senescence-associated secretory phenotype (SASP). The SASP of tumour cells can activate the tumour immune response and promote the clearance of senescent tumour cells (senescence surveillance). We have found that tumour-infiltrating myeloid cells can hinder senescence surveillance by inhibiting the proliferation and function of T cells. In these tumours, the senescent tumour cells through the SASP can support tumour growth by increasing angiogenesis, cell migration, invasion and even metastasis. The aim of our research is to selectively eliminate the senescent cells. This approach known as senolytic therapy has been proposed as a strategy to improve the efficacy of currently available treatments in tumours where immunosuppression hinder the clearance of senescent cells. Nowadays, there are few examples of effective senolytic compounds for cancer therapy. The majority of these compounds target Bcl-2, a regulator of apoptosis that is found increased in senescent cells. However, the efficacy of Bcl-2 inhibitors is variable and it depends on the genetic background of the senescent tumour cells being effective in certain type of senesce response but not in others. The main objectives of our work are 1) Identification of pro-senescent compounds in PTEN null prostate tumours 2) Development of novel senolytic therapies for chemotherapy-treated prostate cancers and 3) Combination of pro-senescent or senolytic compounds with cheek point inhibitors.

131

Anti-Human CD117 CAR T-Cells Efficiently Eliminate Hematopoietic Stem and CD117-Positive AML Cells

R. Myburgh (1), J. Kiefer (2), N. Russkamp (1), S. Surema (1), M. Wilk (1), A. Müller (1), M. Van den Broek (1), B. Burkhard (1), D. Neri (2), M. G. Manz (1)

(1) University of Zurich, (2) ETH, Zurich

Introduction: Acute Myeloid Leukemia (AML) originates from immature hematopoietic stem and progenitor cells (HSPC). While some AML are curable, disease relapse occurs in most of patients upon application of current standard chemotherapy approaches. Recently, eradication of leukemia or lymphoma cells by immunologically targeting lineage specific surface antigens (e.g. CD20, CD19, BCMA) has been achieved. This approach, however, accepts collateral eradication of healthy counterpart lymphoid tissue, which subsequently regrows from HSPCs. To date, the search for AML-specific surface antigens has remained largely elusive. We thus propose in a proof of concept to target the HSPC antigen c-Kit (CD117) expressed by healthy HSPC as wells as by leukemic blasts in >90% of AML patients with CD117 specific CAR T cells, terminate the response, and subsequently conduct healthy/allogeneic HSC transplantation.

Methods: We generated a lentiviral vector which incorporates the CAR (scFv linked to intracellular CD3ζ and 4-1BB signaling domains via stalk and transmembrane regions derived from CD8), followed by a T2A ribosomal skip sequence and RQR8 as selection marker and depletion gene (surface expression of CD34 and CD20 epitopes). The scFv was extracted from a previously published bivalent anti-CD117 antibody (clone 79D) that was derived from a human phage library (Reshetnyak et al., PNAS, 2013). 79D exhibits high binding affinity to an epitope in the membrane-proximal domain of human CD117. Human CD117 was cloned in human CD117 negative HL-60 AML cells and cell lines with stable expression of CD117 at various levels were derived from these.

Results: T-cells were isolated from healthy donors or AML patients in complete remission, respectively, and exhibited sustained growth after activation with recombinant human IL-2 and CD3/CD28 beads. Lentiviral transduction yielded consistently high transduction rates, ranging from 55 – 75% .In co-culture assays, CAR T-cells eliminated more than 90% of $CD117^{high}$ leukemia cell lines within 24 hours at effector-to target ratios (E:T) of 4:1 and 1:1 and more than 50% at E:T

of 1:4. CAR-mediated cytotoxicity correlated with levels of CD117 surface expression as the elimination of $CD117^{low}$ target cells was less efficient compared to $CD117^{high}$ and $CD117^{intermediate}$ cells. In long–term cytotoxicity assays (45d), only $CD117^{low}$ cells were able to escape CAR-mediated killing. In the setting of primary cells, anti-CD117 CAR T-cells effectively depleted >90% of lin-$CD117^{+}CD34^{+}CD38^{+}$ and >70% of lin-$CD117^{+}CD34^{+}CD38^{-}$ cells from healthy bone marrow in vitro within 48 hours. Similarly, >70% of patient derived leukemic blasts were eliminated by autologous anti-CD117 CAR T-cells within 48 hours (1:1 ratio of CAR T cells:blasts). No outgrowth of leukemic blasts was observed in the presence of autologous CAR T-cells over 3 weeks. To determine effectivity of anti human CD117 CAR T-cells in vivo, humanized mice were engrafted with umbilical cord blood $CD34^{+}$ cells. A single injection of $2x10^{6}$ anti-CD117 CAR T-cells resulted in >90% depletion of $CD117^{+}$ cells in the bone marrow within 6 days. Finally, humanized mice transplanted with primary $CD117^{+}$ AML were treated with patient-derived autologous CAR T-cells. At 6 weeks after injection of CAR T-cells, >98% of hu-CD45 $CD117^{+}$ cells were depleted in the bone marrow while control human T-cell treated mice showed full-blown $CD117^{+}$ AML.

Conclusions: We provide proof of concept for the generation of highly-potent CAR T-cells re-directed against CD117 from healthy human donors and AML patients. Anti-CD117 CAR T-cells exhibit high cytotoxic activity against $CD117^{+}$ cell lines as well as primary healthy HSPC and patient AML cells in vitro and in vivo in murine xenograft models. Strategies for the complete elimination of CAR T-cells (immunologic or small molecule based) are required before translation of this approach to the clinical setting.

132

Defining the cell populations responsible for skin cancer initiation and relapse following therapy

A. Sánchez-Danés, J. C. Larsimont, M. Liagre, C. Blanpain

Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles, Belgium

The identification of specific cell type from which cancer arises and the cancer cell population that resists upon therapy leading to tumor relapse constitute the main topics of our research. We use the basal cell carcinoma (BCC), the most frequent cancer in humans, as a cancer model for our studies.

To uncover the cancer cell of origin in BCC and the changes in the cellular dynamics that lead to tumor initiation, we assessed the impact of oncogenic hedgehog signalling activation in distinct cell populations and their capacity to induce BCC. We found that only stem cells, and not progenitors, were competent to initiate tumour formation upon oncogenic hedgehog signalling. Interestingly, this difference was due to the hierarchical organization of tumour growth in oncogene-targeted stem cells, characterized by an increase of symmetric self-renewing divisions and a higher p53-dependent resistance to apoptosis, leading to rapid clonal expansion and progression into invasive tumours [1].

To study the cancer cell population that mediates BCC relapse upon therapy, we treated two different genetic BCC mouse models with a Smoothened inhibitor (Smoi), the most commonly drug used to treat locally advanced and metastatic BCC. The mechanisms by which Smoi leads BCC regression and emergence of resistant tumor cells are currently unknown. We found that Smoi mediates tumour shrinkage by promoting the terminal differentiation of the tumour cells. During the course of Smoi administration, some BCCs become resistant to Smoi therapy, mimicking the situation found in humans. We found a population of persistent slow-cycling tumour cells expressing Lgr5 and characterised by active Wnt signalling that mediates resistance to Smoi, in both mice and humans. Upon treatment discontinuation, this Lgr5-expressing tumour persistent population leads to BCC relapse. Lgr5 lineage ablation or Wnt signalling inhibition together with Smoi results in BCC eradication. Altogether, our study reveals that Smoi induces tumour regression by promoting tumour differentiation, and demonstrates that the synergy between the Wnt and Smo inhibitors constitutes a clinically relevant strategy to overcome resistance to therapy and prevent BCC relapse [2].

1. Sánchez-Danés A, Hannezo E, Larsimont JC, Liagre M, Youssef KK, Simons BD, Blanpain C. Defining the clonal dynamics leading to mouse skin tumour initation. Nature. 2016 Aug 18;536(7616):298-303. doi: 10.1038/nature19069

2. Sánchez-Danés A, Larsimont JC, Liagre M, Muñoz-Couselo E, Lapouge G, Brisebarre A, Dubois C, Suppa M, Sukumaran V, Del Marmol V, Tabernero J, Blanpain C. A slow-cycling LGR5 tumour population mediates basal cell carcinoma relapse after therapy. Nature. 2018 Oct;562(7727):434-438. doi: 10.1038/s41586-018-0603-3.

133

Potential of the water channel aquaporin 1 for detecting migrating tumor cells

M. Lomora (2), Z. Huo (1), U. Kym (1), S. Holland-Cunz (1), S. Gros (1)

(1) University Children's Hospital Basel, Department of Pediatric Surgery, Basel, Switzerland, (2) Department of Physical Chemistry, University of Basel, Basel, Switzerland

Neuroblastoma is the most common solid tumor in children outside the central nervous system. Moreover, it is characterized by a high inter- and intra-tumor heterogeneity. Spread of neuroblastoma cells to the bone marrow is well established and part of staging and choice of therapeutic strategy. But not much is known about the role of circulating tumor cells in this disease. The water channel protein aquaporin 1 (AQP1) has been implicated in mechanisms of tumor cell migration and agility through alterations in cell water permeability. The aim of this study was to investigate whether hypoxia influences AQP1 expression in neuroblastoma cells, how AQP1 expression influences functional and migratory behaviour of cells and to further characterize properties of migrating neuroblastoma cells in search of an efficient marker for detecting migrating tumor cells in neuroblastoma.
AQP1 expression was measured under normoxic and hypoxic conditions by IHC, western and quantitative PCR. Confocal microscopy was used to visualize cell permeability of human neuroblastoma cells and was quantified using stopped flow analysis. For assessment of AQP1 dependent cell motility AQP1 knockdown cells were compared to AQP1 positive cells. Properties of migrating tumor cells were characterized using a modified transwell assay and quantitative PCR.
AQP1 expression increased under hypoxic conditions. The reaction of neuroblastoma cells to the osmotic gradient showed significant differences dependent on their AQP1 expression. This effect was visualized by confocal microscopy. Cell motility showed significant differences depending on different levels of AQP1 expression. Migrating tumor cells expressed significantly increased levels of AQP1 amongst other hypoxia-induced factors.
Hypoxia and through it changed levels of AQP1 expression modify cell water permeability and motility and thus influence the ability of neuroblastoma cells to move. The potential of tumor cells to adapt their behavior with changing AQP1 expression might significantly contribute to tumor cell migration. Through further characterization of migrating tumor cells we found the hypoxia-induced water channel aquaporin 1 to be a potential marker candidate for efficiently detecting migrating tumor cells in neuroblastoma.

134

Hidden diversity in the leukocyte landscape of human brain cancer

E. Friebel (1), K. Kapolou (2), N. G. Núñez (1), S. Tugues (1), M. Weller (2), M. C. Neidert (2), B. Becher (1)

(1) Institute of Experimental Immunology, University of Zurich, Zurich, (2) Laboratory of Molecular Neuro-Oncology, Department of Neurology, Clinical Neuroscience Center, University Hospital Zurich and University of Zurich

The tumor microenvironment (TME) is formed by the interaction of tumor, stromal and various immune cells. In the context of brain cancer, the immune compartment contains resident phagocytes (microglia) as well as tissue-invading leukocytes. Our study had the goal to identify whether the nature of the TME in brain cancer is shaped by the tissue or by the cancer type.
To address this question, we performed a single-cell mass cytometry analysis of primary (including glioblastoma) and secondary (metastasis arised from melanoma and NSCLC) brain cancer tissue samples. The high-dimensional analysis allowed us to identify the distribution of major (microglia/macrophages and T cells) and minor immune populations of the TME (such as NK and B cells, dendritic cells and neutrophils). We observed that the immune compartment of glioma lesions was mainly represented by microglia. In contrast, up to the half of immune cells in metastatic lesions was microglia and monocytes-derived macrophages (MDMs), the other half was T lymphocytes. Moreover, an extensive characterization of myeloid populations revealed the reactive phenotype of microglia and the pro-tumor phenotype of MDMs, which were correlated with the type of tumor.
The complex analysis showed variations in the immune cell composition of the TME, which depends on the tumor origin. We believe that our experimental approach can be applied to following studies of brain cancer to stratify glioma from metastatic tumors and this information could be used as potential targets for immunotherapy.

135

Studying the Molecular Basis of Oncogene-Induced DNA Damage

A. Mihai, P. Janscak

IMCR, University Zurich, Zurich

Genomic instability is a hallmark of cancer. A major cause of genomic instability is oncogene-induced replication stress that drives the transformation of precancerous lesions into malignant tumors. Activated oncogenes have been shown to cause such stress by promoting head-on collisions between the transcription and replication machineries that can lead to the formation of co-transcriptional R-loops, a potent block to replication fork progression. R-loops are three stranded nucleic acid structures that are formed when the nascent RNA released from a transcribing RNA polymerase re-anneals with the template DNA strand, leaving the non-template DNA strand in single-stranded form. To date, there has been no clear link established between oncogene-induced replication stress and the formation of R-loops. Additionally, there has been little research towards identifying and characterizing the factors that are responsible for replication fork progression in cells subjected to oncogene-induced replication stress. Based on current research conducted in our laboratory, a model for resolution of transcription-replication conflicts has been proposed. In the case of R-loop-associated transcription-replication collisions, MUS81-EME1 endonuclease triggers replication fork restart when poly (ADP-ribose) polymerase inhibitors (PARPi) are added to suppress the fork reversal pathway. For our experiments, we chose primary non-immortalized BJ fibroblasts that inducibly overexpress different oncogenes. We could show that replication fork speed decreases upon Cyclin E overexpression. This replication fork slowing phenotype could be rescued by PARP inhibition in a manner dependent upon MUS81. The major effect of Cyclin E overexpression is the shortening of the G1 phase. As a result, conflicts between the replication and transcription machineries may arise. We could demonstrate dependence of oncogene-induced replication stress on transcription by finding that transcription inhibition with triptolide rescued Cyclin E-induced replication fork slowing. Our data suggest that R-loop-mediated transcription-replication conflicts are a major cause of replication fork stalling under conditions of oncogene-induced replication stress.

136

Biological role WNT5A in breast cancer inflammation and metastasis

M. Manchanda, T. Andersson

Department of Translational Medicine, Division of Cell and Experimental Pathology, Lund University, Clinical Research Centre, Skåne University Hospital Malmo, Malmo, Sweden

Objective: WNT5A has been implicated in various metabolic and inflammatory disorders. WNT5A act as a tumour suppressor in breast cancer and re-activating this signalling pathway impairs the migratory and invasive potential of triple negative breast cancer cells. The objective of this study was to investigate the biological role of WNT5A signalling on inflammation-driven metastasis of breast cancer cells.

Method: The expression of COX-2 was analysed in different human breast cancer cell lines by Western blotting . Effects of WNT5A and WNT3A on inflammatory signalling molecules such as COX-2 and NFkB1 production were assessed by RT-qPCR and western blotting in COX-2 expressing breast cancer cells (MDA MB- 468 and MDA MB-231 cells). Role of WNT5A in inflammation-driven metastasis of breast cancer cells was confirmed by evaluating the expression of pro-metastatic cathepsin L and B in WNT-5A or WNT5A mimicking peptide, Foxy-5 treated cells after 24 and 48 hours by Western blotting.

Results and conclusion: In breast cancer cells, treatment with WNT5A significantly reduced the expression of COX-2 while WNT3A increased the expression of COX-2. WNT5A treatment in triple-negative breast cancer cells resulted in reduced levels of cathepsin L and B. Similarly, invasive breast cancer cells on treatment with a WNT5A-mimicking peptide, responded by decreasing the expression of both cathepsin L and B. These data suggest that the anti-metastatic effect of WNT5A in breast cancer might be mediated by decreased levels of cathepsin L and B and could be used as therapeutic targets in patients treated with the WNT5A agonist.

137

Role of co-transcriptional R-loops in G1 phase of the cell cycle

Z. Nascakova (1), B. Boleslavska (1), J. Dobrovolna (1), P. Janscak (2)

(1) Institute of Molecular Genetics, AS CR, Prague, (2) Institute of Molecular Cancer Research, University of Zurich, Zurich

RNA:DNA hybrids, referred to as R-loops, are three-stranded nucleic acid structures formed by nascent RNA annealed to the transcribed DNA strand, with the non-transcribed DNA strand exposed as a single-stranded DNA loop, which is vulnerable to breakage and nucleotide modifications. R-loops have been shown to be involved in various physiological processes including immunoglobulin class switch recombination and telomere maintenance. R-loops are potentially hazardous structures, as they act as a factor contributing to the transcription-replication conflicts and can cause replication stress if they accumulate in an uncontrolled way in the genome. Although it is well established that unscheduled formation of R-loops is strongly genotoxic, the exact mechanisms and conditions leading to R-loop formation are unclear.

We have established a molecular reporter to study R-loop formation in cells. It is comprised of a stably integrated cassette inducibly-expressing a catalytically-inactive mutant of RNase H1 fused with green fluorescent protein [RNH1(D210N)-GFP]. This mutant form of RNase H1 can recognize and bind to R-loops, but due to inactivating point mutation in the nuclease catalytic site is not able to cleave the RNA moiety within the hybrid, increasing R-loop stability.

A previous study in yeast has demonstrated that the homologous recombination machinery contributes to the formation of R-loops. We used our reporter to gain a better understanding of molecular mechanisms involved in the formation of R-loops and subsequent genesis of genomic instability. Surprisingly, we found that depletion of the key factors of homologous recombination including RAD51, BRCA1 and BRCA2 as well as inhibition of RAD51 by the B02 inhibitor, did not prevent R-loops formation induced by inhibition of spliceosome assembly (assessed as accumulation of RNH1(D210N)-GFP on chromatin). Therefore, these factors are probably not essential for R-loop formation in human cells. Interestingly, we noticed that treatment of cells with B02 inhibitor resulted in accumulation of R-loops specifically in G1 phase of the cell cycle. Although the function of RAD51 in G1 phase is unknown, it might be involved in processes that indirectly lead to R-loop formation. Our observations suggest that the occurrence of B02-induced R-loops might alter the timing of transition between the cell cycle phases.

138

Identification of ADAR1 adenosine deaminase dependency in a 1 subset of cancer cells

H. S. Gannon (1), T. Zou (1), M. K. Kiessling (3), G. F. Gao (2), P. Choi (2), D. Cai (2), W. C. Hahn (1), M. Meyerson (1)

(1) Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA, (2) Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA, (3) Department of Medical Oncology and Hematology, University of Zurich and University Hospital Zürich, Zürich, Switzerland

Systematic exploration of cancer cell vulnerabilities provides an opportunity for the development of novel cancer therapeutics. Through analysis of genome-scale loss-of-function datasets, we identified adenosine deaminase acting on RNA (ADAR or ADAR1) as an essential gene for the survival of a subset of cancer cell lines. ADAR1-dependent cell lines displayed increased expression of interferon-stimulated genes (ISGs) and produced interferon-β (IFN-β) spontaneously. Activation of type I interferon (IFN-I) signaling in the context of ADAR1 deficiency could also induce cell lethality in non-ADAR1-dependent cancer cell lines. Transcriptome analysis revealed EIF2AK2, encoding the double-stranded RNA (dsRNA)-activated protein kinase, protein kinase R (PKR), as the most highly correlated gene with cellular ADAR1 dependency. ADAR1 deletion resulted in PKR phosphorylation and activation of downstream signaling. Disruption of PKR signaling, through either inactivation of PKR or overexpression of a wildtype (WT) p150 isoform of ADAR1, partially rescued cell lethality after ADAR1 loss. Furthermore, overexpression of a catalytically-inactive mutant ADAR1-p150 also partially rescued cell lethality induced by ADAR1 deletion, suggesting that both catalytic and non-enzymatic functions of ADAR1 may contribute to preventing cell lethality. Taken together, these data nominate ADAR1 as a potential therapeutic target in a subset of cancers.

139

Engineered Humanized Bone Marrow Microenvironments for Investigating Breast Cancer Metastasis In Vivo

Q. Vallmajo-Martin (1), A. E. Chiou (4), M. Whitman (4), I. Martin (3), C. Fischbach (4), M. Lutolf (2), M. Ehrbar (1)

(1) Laboratory for Cell and Tissue Engineering, University Hospital Zürich, Switzerland, (2) Laboratory of Stem Cell Bioengineering, EPFL Lausanne, Switzerland, (3) Department of Biomedicine, University Hospital Basel, Switzerland, (4) Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, USA

Though it is well known that bone is the preferential homing site for breast cancer metastasis, the disease remains largely incurable with only palliative treatment options. This clinical void can be partially attributed to the lack of suitable animal models that mimic key features of metastasis of human tumor cells to the human bone microenvironment. To address this, we designed a humanized tissue engineered bone model that can be subcutaneously implanted in mice. After bone formation, human breast cancer cells were subsequently introduced to the system and their dissemination to the humanized ossicles could be tracked via bioluminescent imaging. Cancerous lesions in the bones could be monitored via microCT and histological evaluation. This sophisticated model holds tremendous promise for further investigation into the factors involved in osteotropic metastasis of breast cancer.

As a first step, the parameters were optimized for maturation of the bone marrow constructs. Polyethylene glycol (PEG) hydrogels (PEG was selected as a defined non-inductive 3D environment) were seeded with various combinations of human mesenchymal stem cells (hMSCs) from diverse sources and bone morphogenetic protein-2 (BMP-2). We first determined the minimum concentration of BMP-2 and minimum cell density for bone marrow-derived hMSCs from healthy donors. Strikingly, we found that 20 million hMSCs/ml without presence of BMP-2 could remodel PEG gels into ossicles after 8 weeks in vivo. MicroCT analysis showed presence of mineralization within the constructs, a characteristic further corroborated by histology. Moreover, these implants not only still contained hMSCs, but were also highly infiltrated by murine osteogenic, endothelial and hematopoietic cells as demonstrated by FACS analysis. When different hMSC sources were compared in this system, it was found that adipose-derived, as well as bone marrow derived hMSCs from osteoporic patients, failed to mineralize in vivo. Results indicated that at a high cell density, bone marrow-derived hMSCs alone can induce bone formation in an inert environment without any inductive factors. Importantly, tissue type and donor health significantly affect hMSC potential.

Taken altogether, this intricate xenograft model featuring humanized bone microenvironments and human tumor cells has potential to be a powerful tool for studying osteotropic metastasis. It can be utilized to study the molecular cargo that may be involved in homing cancer cells to bone, priming the pre-metastatic site for secondary lesions, and mapping out morphological changes in the bone microenvironment that contribute to disease progression. Furthermore, it may prove valuable as preclinical tool for screening novel therapeutics that combat metastatic processes allowing for earlier intervention and vastly improved patient outcomes.

140

Antagonizing functions of SPOP and ERG Drive Incompatible Subtypes of Prostate Cancer

G. El Tekle (1), M. Bolis (1), A. Mutti (1), T. Svinkina (2), M. Zoma (1), A. Rinaldi (1), V. Ceserani (1), H. Janouskova (1), R. Geiger (3), Z. Dong (4), C. Yang (4), A. Rinaldi (1), P. Schraml (5), G. Carbone (1), A. Alimonti (5), T. Bernasocchi (1), H. Moch (5), S. Carr (2), N. Udeshi (2), J. Theurillat (1)

(1) Institute of Oncology Research, Bellinzona, Switzerland, (2) The Broad Institute of MIT & Harvard, Cambridge, (3) Institute of Research in Biomedicine, Bellinzona, (4) State Key Laboratory of Drug Research, Shanghai, (5) Departement of Pathology and Molecular Pathology, Zurich

Driver genes with a mutually-exclusive mutation pattern across tumor genomes are generally thought to have overlapping roles in tumorigenesis. Here we show that highly recurrent but mutually-exclusive prostate cancer driver alterations involving the oncogenic ERG transcription factor and the ubiquitin ligase adaptor SPOP are synthetic sick. At the molecular level, both driver genes inhibit each other in a reciprocal manner and consequently lead to the emergence of incompatible prostate cancer subtypes. In ERG-driven tumors, ERG itself transcriptionally up-regulates wild type SPOP to dampen androgen receptor (AR) signaling and sustain ERG activity in part through its ability to degrade the bromodomain histone reader ZMYND11. Conversely, SPOP-mutant tumors stabilize ZMYND11 to repress ERG-function and enable oncogenic androgen receptor signaling. In line with this, SPOP mutant tumors acquire gene deletions in the

chromatin-modifying enzyme CHD1 to prevent the generation of fatal ERG gene fusions. Taken together, our findings reveal the existence of incompatible prostate cancer pathways with implications for personalized cancer therapy. More generally, the results demonstrate that a mutually-exclusive mutation pattern can result from a synthetic sick interaction of driver genes.

141

Cytotoxic activity of Arsenic trioxide towards cancer drug resistant cell lines via inhibition of transcription factors

M. Dawood, S. Hamdoun, T. Efferth

Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, Germany

Arsenic trioxide is a traditional remedy in Chinese Medicine since ages. Nowadays, it is clinically used to treat acute promyelocytic leukemia (APL) by targeting PML/RARA. However, the drug's activity is broader and the mechanisms of action in other tumor types remain unclear. In this study, we investigated molecular modes of action by classical and network pharmacological approaches. CEM/ADR5000 resistance leukemic cells were similar sensitive to As_2O_3 as their wild-type counterpart CCRF-CEM (resistance ratio: 1.88). Drug-resistant U87.MG ΔEGFR glioblastoma cells harboring mutated epidermal growth factor receptor were even more sensitive (collateral sensitive) than wild-type U87.MG cells (resistance ratio: 0.33). HCT-116 colon carcinoma $p53^{-/-}$ knockout cells were 7.16-fold resistant toward As_2O_3 compared to wild-type cells. Forty genes determining cellular responsiveness to As_2O_3 were identified by microarray and COMPARE analyses in 58 cell lines of the NCI panel. Hierarchical cluster analysis-based heat mapping revealed significant differences between As_2O_3 sensitive cell lines and resistant cell lines with p-value: 1.86×10^5. The genes were subjected to Galaxy Cistrome gene promoter transcription factor analysis to predict the binding of transcription factors. We have exemplarily chosen NF-κB and AP-1, and indeed As_2O_3 dose-dependently inhibited the promoter activity of these two transcription factors in reporter cell lines. Furthermore, the genes identified here and those published in the literature were assembled and subjected to Ingenuity Pathway Analysis for comprehensive network pharmacological approaches that included all known factors of resistance of tumor cells to As_2O_3. In addition to pathways related to the anticancer effects of As_2O_3, several neurological pathways were identified. As arsenic is well-known to exert neurotoxicity, these pathways might account for neurological side effects. In conclusion, the activity of As_2O_3 is not restricted to acute promyelocytic leukemia. In addition to PML/RARA, numerous other genes belonging to diverse functional classes may also contribute to its cytotoxicity. Network pharmacology is suited to unravel the multifactorial modes of action of As_2O_3.

142

BCP- and T-ALL cells hide in distinct niches of the bone marrow

G. Zuchtriegel (1), D. Capron (1), L. Behrmann (1), L. Kunz (4), B. Marovca (1), M. Kirschmann (3), C. Nombela-Arrieta (2), U. Ziegler (3), T. Schroeder (2), B. Bornhauser (1), J. P. Bourquin (1)

(1) Oncology department, University Children's Hospital, Zurich, (2) Hematology and Oncology, University Hospital Zurich, (3) Center for Microscopy and Image Analysis, University of Zurich, (4) ETH Zurich (D-BSSE), Basel, Switzerland

Relapse arising from residual blasts that survive chemotherapy in the bone marrow is a major problem in the treatment of pediatric acute lymphoblastic leukemia (ALL). In this context, the role of specific bone marrow compartments for the survival of leukemic cells remains incompletely understood. We have established a xenotransplantation model to monitor interactions of human patient-derived leukemia cells with components of the bone marrow microenvironment, in particular after multiagent induction chemotherapy. Using confocal 3D imaging of the bone marrow, B-cell precursor (BCP)-ALL cells were detected in a perivascular niche in close proximity to bone marrow sinusoids, where they survived chemotherapy. In contrast, T-ALL cells were spread all over the bone marrow preferentially clustering in the endosteal space upon drug treatment. Among the residual chemotherapy-surviving leukemia cells, few cells showed reduced proliferative activity, while they were not completely dormant. The distribution pattern of these leukemic cells with reduced proliferative activity was equivalent to the average distribution of all chemoresistant leukemic cells in the bone marrow, with BCP-ALL cells colocalizing with bone marrow sinusoids and T-ALL cells preferentially accumulating close to the bone. These results show that the bone marrow microenvironment orchestrates the localization of different ALL subtypes in distinct niches where they survive chemotherapy. Our model provides the basis for the identification of molecular signals that provide protection of leukemia cells from chemotherapy in their niche.

143

Structural and functional dynamics of the bone marrow stromal microenvironment after chemotherapy

U. Suessbier (1), A. Gomariz (1), S. Isringhausen (1), P. Helbling (1), T. Nagazawa (2), M. G. Manz (1), C. Nombela-Arrieta (1)

(1) Department of Hematology and Oncology, University Hospital of Zurich, Zurich, Switzerland, (2) Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

As the major site of hematopoiesis, the stepwise generation of mature blood cells of all lineages, the bone marrow (BM) contains the rare population of hematopoietic stem and progenitor cells (HSPCs). To sustain the cell production throughout the entire adult lifespan, the differentiation and proliferation of HSPCs is tightly regulated by intrinsic signals but also by the BM microenvironment, which comprises cells of endothelial, mesenchymal and neural origin, which provides signals through direct cell-cell interactions but also through the release of cytokines.

For many different haematologic and non-haematologic malignancies chemotherapy is the primary therapeutic approach. In this context, 5-fluorouracil (5-FU), which targets cycling cells by inhibiting DNA replication, is one of the most commonly used drugs especially in the treatment of colorectal cancer. Furthermore, it is extensively used to investigate the effect of myeloablative treatments. In comparison, the structural effects, especially the kinetics of the destruction and the recovery, on the BM microenvironment and its different components are less characterized.

In our study we provide a comprehensive analysis of the changes in the BM upon 5-FU treatment through two complementary approaches. Quantitative changes of the hematopoietic but especially of the stromal compartment up to 56 days after 5-FU administration, were studied using advanced flow cytometric protocols. In addition, structural effects on the murine BM microenvironment were visualized and quantified using 3D-confocal microscopy imaging of thick bone slices in combination with computational tools that have been developed in our group. In addition, CXCL12-GFP knock-in mice were utilized to gain a better insight in the damage and recovery of the densed reticular stromal cell network.

Lin$^-$c-kit$^+$Sca-1$^-$ and Lin-c-kit$^+$Sca-1$^+$ cells, as well as endothelial cells, phenotypically defined as CD45-Ter119-Sca1$^+$CD31$^+$, were severely depleted 7 days upon 5-FU treatment, but recovered to normal cell counts within 14 to 28 days post 5-FU administration. This reduction was accompanied by a complete destruction of the BM microenvironment, especially of the vascular system. Initially a massive sinusoidal vasodilation was noted, followed by a complete destruction of the vessel wall integrity. Mostly proliferating sinusoidal endothelial cells led to the recovery of cell numbers and reorganization of the vascular network that completely recovered by day 28. In contrast, CXCL12 abundant reticular stromal cells were only slightly affected by the treatment, shown by morphological changes but they were not depleted based on the quantitative analysis of our 3D-images.

Our results demonstrate the regenerative potential of the BM microenvironment to completely recover after severe tissue damage, which is highly robust. Further studies after repeated challenge of the system through three consecutive treatments with 5-FU, which better reflects the clinical setting, showed similar kinetics of the recovery post chemotherapy.

144

Inflamm-aging of Hematopoietic Stem Cells

L. V. Kovtonyuk (1), H. Takizawa (2), M. G. Manz (1)

(1) Department of Hematology, University Hospital Zurich and University Zurich, (2) Kumamoto University, Kumamoto, Japan

Lifelong blood production is sustained through a stepwise differentiation program by rare self-renewing Hematopoietic Stem Cells (HSCs) in the bone marrow (BM). With aging HSCs show reduced self-renewal, less efficient bone marrow (BM)–homing capacity, and myeloid-skewed differentiation. We here tested how extrinsic and intrinsic factors determine HSC behaviour during aging.

CFSE-labelled young and aged LKS cells were each transferred into steady state, non-irradiated young and aged WT mice. BM analysis at 8 weeks after divisional tracking showed that young HSPCs proliferated faster than old HSPCs. Moreover, both young and old HSPCs were relatively more dormant in an old versus a young environment, indicating an increased intrinsic and extrinsic drive towards quiescence during ageing. To test HSCs function, we isolated quiescent and cycling LKS fractions from various combinations and transplanted them into lethally irradiated mice. Mice were monthly bled to follow long-term donor engraftment and lineage repopulation. Quiescent aged donor HSCs, isolated from young or old recipients, favoured myelopoietic differentiation. Similarly, cycling aged HSC isolated from aged environment showed myeloid biased repopulation. In contrast, cycling aged HSCs that were exposed to a young environment showed

balanced lineage repopulation (similar to young HSCs that were cycling within a young or an aged environment). By looking for relevant environmental factors we found increased expression of RANTES, MIP-2, IL-1α and IL-1β in the aged environment. CFSE-dilution and functional HSC read-outs revealed that IL-1α and IL-1β drive young HSC towards proliferation, while this effect is mitigated in aged HSCs, despite the upregulated expression of IL1RI on aged HSCs. Moreover, analysis of aged IL1RI KO mice revealed a reduced aging-associated HSCs phenotype and improved lymphoid lineage repopulation upon transplantation into lethally irradiated mice.
Our data demonstrate that proliferative history imprints a cell-intrinsic dormancy program on HSCs, which is associated with myeloid-biased differentiation and, at least in natural ageing, with increased IL-1 signalling. Interestingly, this HSC program can be partially rejuvenated upon cycling, but not upon dormancy, in young steady-state environments.

145

Loss of ganglioside glycosyltransferase SIAT8 results in mesenchymal-to-epithelial transition in ovarian cancer cells

C. Cumin (1), Y. L. Huang (1), N. Rimmer (1), C. Y. Liang (1), M. Nunez Lopéz (1), A. Fedier (1), V. Heinzelmann-Schwarz (2), F. Jacob (1)

(1) Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland, (2) Hospital for Women, Department of Gynecology and Gynecological Oncology, University Hospital Basel and University of Basel, Switzerland

Each eukaryotic cell surface contains a repertoire of glycosphingolipids (GSLs) which consists of three major series: globo, (neo-)lacto, and ganglio series. We have recently demonstrated, by genomic deletion of the globoside key glycosyltransferase A4GALT, that globoside GSLs trigger reversible Epithelial-to-Mesenchymal transition (EMT) in ovarian cancer [1]. In contrast, ganglioside glycosyltransferase-encoded genes are generally upregulated in ovarian cancer patients with mesenchymal features. Thus, we hypothesize that genomic deletion of ganglioside glycosyltransferase encoding genes will induce MET.
Bioinformatic analysis of publicly available ovarian cancer transcriptomic data sets identified ST8SIA1 (SIAT8) as potential candidate for MET. Therefore, we deleted the SIAT8 gene (ΔSIAT8) by targeting his open reading frame by using our paired sgRNA approach and the CRISPR-Cas9 technology [1,2]. Beside altered cell morphology, we observed MET-like changes in vitro (e.g.reduced cell motility, increased spheroid formation and cell proliferation). On the molecular level, we observed reduced Vimentin, Snail and Slug expression accompanied with increase of epithelial cell markers such as E-cadherin and Claudin 1. Importantly, we were also able to rescue this EMT marker changes by lentiviral transduction of ΔSIAT8 cells for bicistronic expression of mCherry and SIAT8.
Our data suggest that the major GSL series play an important role in reversible Epithelial-to-Mesenchymal transition. In order to elucidate the molecular pathways linking GSLs to EMT/MET, we are currently utilizing the CRISPR activation (CRISPRa) library approach. Moreover, we aim to translate our cell line-based findings by testing GSLs and SIAT8 together with classical EMT marker expression in ovarian cancer tissue samples.

1. Jacob et al., 2018 Cancer Research 78(11): 2952-2965
2. Alam et al., 2017 Scientific Reports 7: 45367

146

Characterisation of novel histone ubiquitinating enzyme involved in chromatin ubiquitination and genome stability

V. Mohana Natarajan, L. Penengo

Institute of Molecular Cancer Research, University Zurich, Zurich

The “mutator phenotype hypothesis” states that genomic instability is seen in precancerous lesions thereby prompting tumour development. The identification of mutations in DNA repair genes in hereditary tumors provides a strong proof of this. The DNA double stranded (DSBs) breaks are among one of the potent threats to the maintenance genome stability. To deal with this, cells elicit a complex response, the DNA damage response or DDR, triggered by protein kinases and leading to the hierarchical accumulation of signalling and repair factors, which are largely under the control of reversible protein ubiquitination on the chromatin surrounding the DSB sites. Key players in this process are the ubiquitin ligases RNF8 and RNF168, which ubiquitinate H2A type histones and promote the accumulation of 53BP1 and BRCA1. These factors are responsible to activate downstream events for DSB repair, either by non-homologous end joining (NHEJ) or homologous recombination (HR), respectively. An additional ubiquitin ligase, RNF169, has been shown to finely regulate this process by limiting the accumulation of 53BP1 at the damaged chromosomes, thereby inhibiting NHEJ.

Recently our lab uncovered an essential new function of these canonical DDR factors – RNF8/RNF168/53BP1 – in regulating DNA replication during unperturbed cell growth. Although RNF169 has been reported to be localized at the replication forks upon genotoxic stress, neither its function in DNA replication nor its targets have been investigated so far. We obtained preliminary data indicating histone H2B as potential substrate of RNF169. By using mouse embryonic stem cells, we found that RNF169 expression is modulated during cell differentiation and correlates with H2B ubiquitination. Since it has been reported that loss of H2B ubiquitination leads to the replication fork stalling and the replisome becomes unstable in the presence of HU, the aim of my project is to investigate the role of RNF169 – via ubiquitination of histone H2B – during DNA replication and replication stress. Ultimately our goal is to identify new factors of DNA repair and replication control to be exploited as therapeutically druggable targets to potentiate chemotherapy.

147

A robust classification of hepatocellular carcinoma based on bimodally expressed genes

L. D. Lukas (1), J. Juliane (1), L. Quagliata (2), L. M. Terracciano (2), M. Heikenwälder (3), A. Weber (1)

(1) Department of Pathology and Molecular Pathology, University Hospital of Zurich, Switzerland, (2) Institute of Pathology, University Hospital of Basel, Switzerland, (3) Division of Chronic Inflammation and Cancer, German Cancer Research Center, Heidelberg, Germany

Hepatocellular carcinoma (HCC) is recognized to be a highly heterogeneous disease. However, previously proposed molecular classifications of HCC have hardly found widespread use in clinical practice. Based on observations in murine and human HCC, we chose to focus on genes with a bimodal pattern of expression ("on-or-off switch" genes), which lend themselves to classification. In a combined dataset of 1821 HCC patients, consensus clustering of bimodally expressed genes revealed 3 subgroups of HCC: The Pericentral subgroup, strongly associated with β-catenin mutations; the Liver Progenitor subgroup, associated with reduced survival and high serum α-fetoprotein expression, and the Other subgroup, showing neither characteristic. We then developed a simple classifier based on 30 bimodal genes which is independent of the method of transcript detection, and corroborated it in novel HCC cohorts. A subset of these genes was further used to develop an immunohistochemical classifier, which can be easily integrated into routine clinical practice. The hereby described classifier allows prognostic stratification and may provide a basis for examining subtype-specific response to therapy.

148

Systematic Analysis of EGFR and HER2 αC-β4 loop insertion mutations

Hannah L. Tumbrink (1,2,3), Martin L. Sos (1,2,3)

(1) Molecular Pathology, Institute of Pathology, University of Cologne, Germany, (2) Department of Translational Genomics, Medical Faculty, University of Cologne, Germany, (3) Center for Molecular Medicine Cologne, University of Cologne, Germany

Targeted therapy has improved non-small lung cancer (NSCLC) patient survival e.g. in EGFR and HER2 mutated patients. However, while the most common EGFR mutants (deletions in exon 19 and mutations encoding pL858R) and HER2 respond well, a relevant proportion present with Exon 20 insertion mutation is resistant.
A better understanding of the molecular effects of Exon 20 insertion mutations is crucial to develop targeted therapies. To study the aberrant EGFR and HER2 kinase activity harbouring Exon 20 mutations we performed comprehensive analysis of the specific mutations by investigating the individual characteristics of activations, regulation and signal transduction. The final goal is, to identify vulnerabilities in Exon 20 mutated cancer types. To this end we generate a large set of Ba/F3 cell lines and NIH3T3 cell lines carrying the most prominent Exon 20 mutations in NSCLC. We compared the growths rate, the respond to tyrosine kinase inhibitors (TKIs) as well as the downstream phosphorylation state of the generated model cell lines with rare patient derived cell lines.
In accordance with clinical observations the cell lines did not respond to first generation inhibitors like erlotinib or third generation inhibitors like osimertinib. On the other hand, second generation EGFR inhibitors like afatinib and poziotinib showed inhibition of the downstream signalling of Exon 20 mutant cells but also had a strong activity on wild type EGFR, thus strongly limiting their clinical use. Thus, the generation of Exon 20 mutated cell lines enables us to study the different behaviour of each mutation and to test novel inhibitors.

149

Evaluation of efficacy and safety of retreatment or concurrent immunoradiotherapy after progression in metastatic melanoma patients previously treated with nivolumab

Y. Nonomura (1), O. Eichhoff (1), M. Nomura (3), A. Otsuka (2), M. Levesque (1), R. Dummer (1)

(1) Department of Dermatology, University Hospital of Zurich, Switzerland, (2) Department of Dermatology, Kyoto University Hospital, Kyoto, Japan, (3) Department of Therapeutic Oncology, Kyoto University Hospital, Kyoto, Japan

Background: Immunotherapy and targeted therapies have improved the prognosis of patients with metastatic melanoma. The objective of this study was to evaluate (1) the efficacy and safety of retreatment with nivolumab and (2) that of concurrent immune checkpoint inhibitor therapy and radiotherapy (immunoradiotherapy) in patients with metastatic melanoma after progression on nivolumab.

Patients and methods: (1) A retrospective review was performed on eight consecutive metastatic melanoma patients retreated with nivolumab who progressed on previous nivolumab. These patients received nivolumab 2 mg/kg every 3 weeks. Best responses to each treatment were assessed using RECIST 1.1. (2) A retrospective review was performed on 16 consecutive patients with metastatic melanoma treated with concurrent immunoradiotherapy after progression on nivolumab. Best responses to immunoradiotherapy were assessed either inside or outside of the radiation fields. The target lesions ratio (the sum of the diameters of the target lesions inside the irradiated fields/all target lesions) was also assessed.

Results: (1) Three out of eight patients received chemotherapy before first nivolumab. The median first nivolumab treatment period was 4.1 months. Three (37.5%) patients achieved a partial response and three (37.5%) patients achieved stable disease as their best response. Between first and second nivolumab, patients were treated with ipilimumab (n = 6), vemurafenib (n = 1), or no other medical treatment (n = 1). Four patients received radiation therapy. The median second nivolumab treatment period was 4.3 months. Two (25%) patients who received second nivolimab achieved a partial response and three (37.5%) patients achieved stable disease as their best response. Among the four patients treated with ipilimumab and radiotherapy between first and second nivolumab, the response rate was 50% and the disease control rate was 75%.

(2) Among the patients, seven received ipilimumab and radiotherapy (Ipi-RT), six received nivolumab and radiotherapy (Nivo-RT), and three sequentially received Ipi-RT and Nivo-RT. As shown in Table 1, the overall response rate (all patients regardless of inside or outside radiation fields) was 30%. The response rate inside the radiation fields was 68.8% for all patients combined. The response rates of Ipi-RT and Nivo-RT inside the radiation fields were 37.5 and 100% (P = 0.03), respectively. Grade 3 adverse events were observed in three patients treated with Ipi-RT. The target lesions ratio was a predictive marker of disease control rate among patients treated with Nivo-RT.
Conclusions: This study showed that retreatment with nivolumab or concurrent immunoradiotherapy is an option for patients with metastatic melanoma after progression on nivolumab.

	All			Nivo-RT			Ipi-RT		
	All	Inside	Outside	All	Inside	Outside	All	Inside	Outside
CR	1	1	1	0	0	0	1	1	1
PR	5	10	0	5	8	0	0	2	0
SD	2	4	7	1	0	6	1	4	1
PD	12	1	12	4	0	4	8	1	8
NE	0	4	0	0	2	0	0	2	0
Response rate (%)	30.0	68.8	5.0	50.0	100.0	0.0	10.0	37.5	10.0
Disease control rate (%)	40.0	93.8	40.0	60.0	100.0	60.0	20.0	87.5	20.0

Table 1. Efficacy of concurrent immunoradiotherapy

150

Dissection and comprehensive analysis of adenosquamous carcinoma in the lung

A. Krause, M. De Filippo, S. Piscuoglio, C.K. Ng, L. Bubendorf

Institute for Pathology, University Hospital Basel, Basel

Background: Acquiring constantly new mutations is one of the key elements in tumor development. There are tumors with unknown origin as the adenosquamous carcinoma (ASC). ASC is a rare subtype of non-small cell lung cancer and consists of an adenocarcinoma (AC) component and a squamous cell carcinoma component (SCC). In this study, we applied a comprehensive approach, including whole exome sequencing (WES) and RNA sequencing to understand the origin and evolutionary relationship between the AC and SCC components of an ASC of the lung.

Methods: FFPE tissue of three ASC patients was used. In each case, samples were stained with FastRed. Both components, AC and SCC, were scratched separately from histological slides for DNA and RNA extraction. WES sequencing was used for calling mutations and copy number aberrations (CNA).

Results: The mutational profile of the AC and SCC components revealed a common trunk of 118 mutations including driver mutations as EGFR exon 19 deletion or TP53 nonsense mutation, alluding a common clonal origin of the components. AC had more private mutations than SCC (234 vs. 134). Unique potential driver mutations where also detected in the branches as TP53 and AKT1 mutations in the AC and TLX1 mutation in the SCC. CNAs were very similar, indicating most CNAs as an early event in tumorigenesis. Truncal events showed high similarity with private mutations of AC in mutational signature. In contrast, private mutations in SCC had a distinct pattern. We assume that the common ancestor is coming rather from AC than SCC. Differently expressed pathways were seen in the transcriptomic profiling. AC showed highly expressed genes, which are related to oxidative phosphorylation, fatty acid metabolism and peroxisome pathways. Contrary, SCC showed genes related to epithelial-mesenchymal-transition, hedgehog signaling and IL6-JAK-STAT3-signaling that were upregulated.

Conclusion: ASC of the lung were macroscopic dissected and analyzed separately. The both different components, AC and SCC, demonstrated clonal relatedness with a different phenotype. For a better understanding of this rare tumor, more samples should be analyzed on a genomic, transcriptomic and epigenomic level.

151

A splicing factor subunit that acts as a co-activator of Hif and contributes to malignancy in pancreatic cancer

P. Simmler (1), C. Cortijo (1), P. Galliker (1), P. Mirtschink (1), C. Müller (3), A. Vukolic (1), G. Pellegrini (2), O. Strobel (4), W. Kovacs (1), W. Krek (1)

(1) Institute of Molecular Health Science, ETH Zurich, Zurich, (2) Institute of Veterinarian Science, University of Zurich, Zurich, (3) Biotech Research & Innovation Centre, University of Copenhagen, Copenhagen, (4) Department of General, Visceral and Transplantation Surgery, Heidelberg University Hospital, Heidelberg

In our research project, we were able to demonstrate that the U2 spliceosome subunit Sf3b1 (splicing factor 3b subunit 1) has a splicing-independent function by acting as a transcriptional co-activator of Hif1a, thereby contributing to pancreatic cancer malignancy.
We show that increased levels of Sf3b1 are correlated with poor survival outcome in patients suffering from pancreatic adenocarcinoma (PDAC), breast cancer and gastric cancer. Also, in various solid human cancer types exists a strong correlation of Sf3b1 and Hif1a, and also of Hif-target genes.
We demonstrate co-immunoprecipitation of Sf3b1 and Hif1a under hypoxia, an Sf3b1-dose dependent VEGFA-promoter activation and also DNA-binding of the splicing factor subunit at promoter regions of various Hif-targets. Altogether, these biochemical assays strongly indicate a yet unreported function of Sf3b1 as co-activator of Hif1a.
To assess the impact of this finding in tumorigenesis, we established a mouse model of PDAC, which is the cancer type that showed the strongest correlation of Sf3b1 and the hypoxia gene signature in human and is widely known to be severely hypoxic.
Our mouse model is driven by pancreas-specific, Cre-mediated activation of KrasG12D and Tp53R172H mutation (KPC). To evaluate the effect of lowered Sf3b1 gene dosage, we introduced Sf3b1 heterozygosity to the well-established KPC mouse model (=KPCS). KPCS mice succumb substantially later to PDAC than KPC mice.
In order to gain deeper insights into the involved downstream mechanisms, we successfully established primary ductal organoid cultures derived from KPC and KPCS mice. The organoid-system allowed us to individually study the ductal cells, from which PDAC likely stems from. KPC and KPCS organoids grow comparable under normoxic conditions, but KPCS organoids, having only 50% Sf3b1 dosage, show impaired growth under hypoxia. This finding strengthen our hypothesis that lowering Sf3b1 levels to a certain extent does not lead to substantial missplicing, likely because there are still enough molecules bound to the spliceosome.
We currently perform several downstream analyses, including RNA-Sequencing, splicing analysis and metabolic assays, aiming at elucidating the exact mechanism of Sf3b1-dosage dependent proliferative impairment. In the next weeks, we would also like to assess if in KPCS cells, there is a lack of unbound Sf3b1 with the potential to interact with Hif1a under low oxygen concentrations. We are confident that this additional data will enable us to elucidate the downstream-mechanism of Sf3b1-dependent Hif1a co-activation.

152

mTOR mediates chemotherapy resistance in KRAS-mutant lung cancer

S. Q. Liang (1), E. D. Bührer (2), S. Berezowska (3), T. M. Marti (1), D. Xu (1), L. Froment (1), H. T. Yang (1), S. R. R. Hall (1), E. Vassella (3), Z. Yang (1), G. J. Kocher (1), M. A. Amrein (2), C. Riether (2), A. F. Ochsenbein (2), R. A. Schmid (1), R. W. Peng (1)

(1) Division of General Thoracic Surgery, Inselspital, Bern University Hospital, Bern, Switzerland, (2) Tumor Immunology, Department for BioMedical Research, University of Bern, Bern, Switzerland, (3) Institute of Pathology, University of Bern, Bern, Switzerland

Oncogenic KRAS mutations comprise the largest subset of lung cancer defined by genetic alterations, but in the clinic no targeted therapies are available that effectively control mutational KRAS activation. Consequently, patients with KRAS-driven tumors are routinely treated with cytotoxic chemotherapy, which is often transiently effective owing to development of drug resistance. In this study, we show that hyperactivated mammalian target of rapamycin (mTOR) pathway is a characteristic hallmark of KRAS-mutant lung adenocarcinoma after chemotherapy treatment, and that KRAS-mutant lung cancer cells rely on persistent mTOR signaling to resist chemotherapeutic drugs. Coherently, mTOR inhibition circumvents the refractory phenotype and restores sensitivity of resistant KRAS-mutant lung cancer cells to chemotherapy. Importantly, drug combinations of clinically approved mTOR inhibitors and chemotherapy drugs synergize in inhibiting cell proliferation of KRAS-mutant cancer cells in vitro and in vivo, and the efficacy of this combination treatment correlates with the magnitude of mTOR activity induced by chemotherapy alone. These results pinpoint mTOR as a mechanism of resistance to chemotherapy in KRAS-mutant lung cancer and validate a rational and readily translatable strategy that combines mTOR inhibitors with standard chemotherapy to treat KRAS-mutant adenocarcinoma, the most common and deadliest lung cancer subset.

153

Proliferative history determines drive to quiescence in hematopoietic stem cells

G. Spaltro, L. Kovtonyuk, M. G. Manz

Department of Hematology and Oncology, University Hospital of Zurich, Zurich, Switzerland

Introduction: Hematopoietic Stem Cells (HSC) sustain lifelong blood production. However, most HSC are quiescent in steady state (>90% in G0 phase of cell cycle). Notably, aged HSCs are more quiescent than young ones, due to altered epigenetic patterns associated with self-renewal and cell differentiation. Previous studies from our group suggest that HSC cycling is regulated by a cell-intrinsic program (e.g. epigenomic imprinting) and by extrinsic signals (e.g. growth factors or pathogen components). While research has focused largely on factors that activate HSC from dormancy, our aim is to enlighten mechanisms that lead dividing HSC to quiescence. We hypothesize that increased proliferative history due to aging and inflammation activates an intrinsic program that drives HSC towards quiescence. Dysregulation of this program in cells with high proliferative history might lead to proliferative exhaustion or accumulation of genetic alterations and HSC malignancies.

Methods: We used a combination of CFSE in vivo labelling (Takizawa et al., J Exp Med, 2011) and Ki-67 proliferation assay to assess HSC cell cycle status after each division (i.e. from 0- to >5-divided cells). We isolated HSC from young (2 months old) or old (2 years old) mice by FACS sorting, labelled them ex vivo with CFSE and transplanted them into young, non-irradiated recipients. After 1-8 weeks, we isolated HSPC from recipient mice and analyzed their proliferative history and cell cycle status by FACS. In addition, we established transplantation of CFSE-labelled human HSPC into NSG and MITRG-SKI mice in order to study the divisional behaviour in the context of human hematopoiesis.

Results: Time-course experiments with young and aged mice show that three weeks after transplantation, all non-divided cells are in G0 phase of the cell cycle. In divided cells we noticed that after each cell division (1-5) a proportion of cells is found in the G0 fraction. These cells seem to remain in G0, since most do not re-enter cell cycle even after 2 months. However, upon external stimuli that mimic viral or bacterial infections, such as Poly I:C and LPS treatment, cells are recruited to cell cycle, demonstrating that the intrinsic drive to

quiescence can be suppressed upon need. In contrast, upon stimulation with thrombopoietin mimetics (Romiplostim) HSC divide and maintain their drive to quiescence after each division, indicating a proliferation burst oriented towards self-renewal. Taken together, these data suggest that the machinery that drive HSCs to quiescence can be fine-tuned upon need in different conditions. When using aged HSC in similar settings, the fraction of non-divided HSC is increased while the number of fast cycling HSC declines. In addition, the amount of spontaneously quiescent HSC after each division is increased, when compared to young HSC. This suggest that a delay in the G0/G1 phase transition might lead to the accumulation of quiescent HSC during aging. In order to mechanistically understand the observed increased drive to HSC quiescence with ageing and proliferative history, we are performing RNA-sequencing in steady-state young and aged HSC as well as in HSC that went through increased cycling upon thrombopoietin treatment as well as serial transplantations. Furthermore, to gain insight in human HSC cycling behaviour, we transplanted CFSE-labelled human CD34+ cells from human fetal liver, cord blood and mobilized HSC from healthy donors into humanized mice and tracked their cycling activity. Results confirm findings in mice that aging is associated with accumulation of quiescent HSC.

Conclusions: Our results seem to confirm our initial hypothesis that increased proliferative history with aging or inflammation drive HSC towards quiescence. This might ensure that the HSC population as a whole goes through a similar overall-turnover at the end of life and therefore might prevent HSC exhaustion and reduce the risk to develop HSC malignancies.

154

CRISPR-mediated activation of MYC paralogs as a framework to discover MYC status-specific biology and vulnerabilities

M. Dammert, M. Sos

Molecular Pathology, Institute of Pathology; Department of Translational Genomics; Center for Molecular Medicine Cologne, University of Cologne, Germany

MYC activation reshapes cellular transcription, promotes cell growth, cell cycle progression and transformation and therefore MYC is considered a strong driver of tumor development. The MYC transcription factor family comprises the paralogs MYC, MYCN, and MYCL that are all associated tumorigenesis. In theory, MYC transcription factors would represent an ideal drug target. However, efforts to directly inhibit MYC transcription factors have failed so far. Therefore, MYC family members are considered undruggable targets. However, potential vulnerabilities associated with the cellular MYC status may provide a basis for therapeutic intervention. Currently, there is a lack of comparative studies assessing the specific contribution of each MYC family member to tumor development. Established cell lines in which MYC family members are overexpressed differ profoundly in their genetic background, thereby impeding the analysis of MYC family member specific effects. Furthermore, the complexity of MYC regulation and MYC biology is not adequately recapitulated by traditional, heterologous MYC overexpression approaches.

We sought to establish an endogenous overexpression system using a CRISPR activation (CRISPRa) approach to activate the individual MYC family members for a systematic comparison of their impact on target gene activation, cell growth and drug sensitivity. Using sgRNAs specific to the promoter of each MYC family member in combination with a nucleolytically inactive Cas9-VP64 fusion protein, we successfully achieved transcriptional upregulation of all three MYC family members in multiple cell lines including NIH3T3 and MEF cells. The increase in MYC mRNA resulted in markedly elevated MYC protein levels and upregulation of canonical MYC target genes like NPM1, FBL, and 45S pre-rRNA demonstrating the functional impact of activated MYC. Moreover, activation of Myc led to increased anchor-independent growth indicating that high MYC levels induce transformation in contrast to MYCN or MYCL. Activation of Myc further led to increased sensitivity to cisplatin and other chemotherapeutic agents.

In conclusion, we established an endogenous Myc-paralog overexpression model system that enables a systematic comparison of the different MYC family members and their specific contribution to tumor initiation, growth and drug sensitivity. The system successfully recapitulated previous observations of MYC-paralog activation and can serve as a platform to probe for MYC family member-specific vulnerabilities and thereby pave the way for novel, targeted therapy approaches in MYC-driven cancer.

155

Modulation of breast cancer stem cells with tumor vs metastasis initiating capacities

F. Fico, C. Rüegg, A. Santamaria-Martínez

Department of Oncology, Microbiology and Immunology, University of Fribourg, Fribourg

Cancer stem cells (CSC) are defined by their ability to regenerate a tumor upon transplantation. However, it is not yet clear whether tumors contain a single CSC population or different subsets of cells with mixed capacities for initiating primary and secondary tumors. Using two different identification strategies, we studied the overlap between metastatic stem cells and tumor-initiating cells in the MMTV-PyMT model. Our results show that in the MMTV-PyMT model, lin-CD90^{-}ALDH^{high}cells retained a high tumor initiation capacity in orthotopic transplants, in contrast to CD24^{+}CD90^{+}, which retain most of the metastatic capacity. Interestingly, suppression of TGFβ signaling increased tumor-initiating cell numbers. We here describe the existence of distinct populations of CSC with differing capacities to initiate tumors in the primary or the secondary site. Inhibiting TGFβ signaling shifts the balance towards the former, which may have unanticipated implications for the therapeutic use of TGF⇓/ALK5 inhibitors.

156

A novel role of MAGI1 as a metastasis suppressor in breast cancer

B. Alday-Parejo, A. Santamaria-Martínez, C. Rüegg

Chair of Pathology, Department of Oncology, Microbiology & Immunology (OMI), Medicine Section, University of Fribourg, Switzerland

MAGI1 is a member of the membrane-associated guanylate kinase (MAGUK) family. Our group has previously shown that MAGI1 plays a crucial role in colon cancer, since its downregulation leads to enhanced Wnt signaling, decreased E-cadherin and disrupted actin stress-fibre and focal adhesion formation.
Human database mining revealed that patients bearing breast tumors with high expression of MAGI1 have a better survival, especially in luminal A and hormone receptor positive breast cancer subtype. Therefore, we hypothesize that MAGI1 plays a role in estrogen-dependent breast cancer subtype and breast cancer metastasis.
In order to study the role of MAGI1 in breast cancer, we performed in vivo studies with two syngeneic models of breast cancer in Balb/c mice: luminal ER+ non-metastatic 67NR cells and triple negative highly metastatic 4T1 cells. MAGI1 is moderately expressed in the non-metastatic cell line 67NR and is expressed at low levels in 4T1 cells. Downregulation of MAGI1 in 67NR and overexpression of MAGI1 in 4T1 renders cells more and less metastatic to the lungs, respectively. In vivo assays indicate that the above-mentioned effects on metastasis are due to differences in extravasation capacity.
We also demonstrated that MAGI1 is a gene upregulated by estrogen and MAGI1 presence is permissive for estrogen receptor signaling in human MCF7 breast cancer cell line. Consequently, downregulation of MAGI1 renders MCF7 cells insensitive to estrogen as they fail to upregulate estrogen-regulated genes such as progesterone and BRCA1. Additionally, MCF7 cells with downregulated MAGI1 proliferate more indicating a more aggressive phenotype.
These results suggest that MAGI1 expression is downregulated upon tumor progression and it is negatively associated with metastatic potential. Moreover, MAGI1 loss upon tumor progression might be related to a more aggressive and proliferative luminal B breast cancer subtype and might predict estrogen receptor signaling independence hence resistance to anti-hormonal therapy.

157

Enhanced support of myelofibrosis stem cells in next generation humanized mice

V. Lysenko (2), N. Wildner (2), K. Zimmermann (2), P. Schürch (2), C. Fritz (1), R. A. Flavell (4), P. J. Wild (3), S. Dirnhofer (5), M. G. Manz (2), A. Theocharides (2)

(1) Institute of Pathology and Molecular Pathology, University Hospital Zurich, Switzerland, (2) Division of Hematology, Department of Hematology and Oncology, University and University Hospital Zurich, (3) Dr. Senckenberg Institute of Pathology, University Hospital Frankfurt, Frankfurt am Main, Germany, (4) Department of Immunobiology, Yale University, New Haven, Connecticut, USA, (5) Institute of Pathology, University Hospital, University of Basel, Switzerland

Introduction: Pre-clinical patient-derived xenograft (PDX) mouse models have emerged as powerful tools for investigating normal and leukemic stem cells (HSCs and LSCs). Successful development of PDX models for aggressive neoplasms, such as acute leukemias, has been

achieved. However, engraftment of less-aggressive neoplasms in PDX models is poor. Myelofibrosis (MF) is a chronic HSC disorder predominantly characterized by an expansion of myeloid cells followed by constant deposition of fibers in the bone marrow (BM) and transformation into AML depending on the clonal evolution of MF stem cells (MF SCs). We hypothesized that the constitutive expression of human cytokines and growth factors in humanized mice (MISTRG, Rongvaux et al., Nature Biotechnology 2014) may provide a supportive microenvironment for MF SC development and could faithfully recapitulate disease phenotype and genetic heterogeneity allowing the development of a pre-clinical MF PDX model.

Methods: Purified peripheral blood (PB) stem and progenitor (CD34+) cells from 13 MF patients were transplanted intra-hepatically into sub-lethally irradiated newborn MISTRG mice and NSG mice (controls). For secondary transplantations, human CD45+ cells purified from primary animals were transplanted intra-hepatically. Mice were sacrificed after 5-26 weeks and characterized by flow cytometry, immunohistochemistry and mutational profiling.

Results:The total median human engraftment was significantly higher in the BM (29.20% vs 4.175%, p<0.0001), the PB (48.70% vs. 0.73%, p<0.0001) and the spleen (8.19% vs. 0.33%, p<0.0001) of MISTRG compared to NSG mice. Overall, the human BM engraftment (more than 2% human CD45 (hCD45)) was observed in all MISTRG mice (49/49, 100%) and 57.5% of NSG mice (23/40). MISTRG mice exhibited superior engraftment independent of risk categories (DIPSS, MIPSS70 and MYSEC), disease stage (chronic, accelerated) and diagnoses (primary, secondary MF). Both NSG and MISTRG mice supported substantial human myeloid (CD33+) engraftment in the BM, PB and spleen. In the BM of both mouse strains, there was an expansion of monocytes and granulocytes in the myeloid compartment. A higher percentage of monocytes to granulocytes was observed in the PB of NSG (p=0.0044) and MISTRG (p<0.0001) mice, while in the spleen, the ratio was higher for only MISTRG (p=0.0254) mice. Next, we characterized the CD34+ hematopoietic stem and progenitor cell compartment. Although, both NSG and MISTRG mice supported an expansion of human CD34 (hCD34) cells out of the hCD45 cell fraction (10.76% vs. 11.07%, p=0.5640), MISTRG mice supported higher engraftment of hCD34 cells out of all the cells (1.044% vs. 2.375, p=0.0168). Furthermore, immunohistochemistry revealed human megakaryocytic differentiation in both strains and reticulin fibers (Gömöri) only in 16.7% (3/18) of MISTRG mice. Since fibrosis is a timely process, we focused on mice that were analyzed after an extended period. Reticulin fibers were detected in 50% (3/6) of MISTRG mice observed over 16 weeks. To determine whether the engrafted human cells were derived from the MF clone, next generation sequencing comparing the mutational profile of primary samples to their corresponding engrafted xenografts was performed. This data showed maintenance of the original clonal composition in the xenografts. Finally, purified human MF cells isolated from primary mice showed significant myeloid reconstitution in secondary recipients.

Conclusions/outlook: Overall, these results show that MISTRG mice support robust engraftment of MF SCs and are able to maintain repopulation capacity and the genetic heterogeneity found in patients. The MF PDX model will further be used to understand the disease pathogenesis and assess established and novel therapeutic agents in order to expedite their transition into clinical trials.

158

EBV-positive Hodgkin lymphoma is associated with reduced NK cell-mediated antibody-dependent cellular cytotoxicity toward EBV-infected B cells

E. Panisova (1), T. Azzi (1), J. Lazarovici (2), S. Bürgler (1), C. Berger (1), D. Ghez (2), D. Nadal (1)

(1) Division of Infectious Diseases and Hospital Epidemiology, University Children's Hospital of Zurich, (2) Department of Hematology, Gustave Roussy and Université Paris Saclay, Villejuif

Natural Killer (NK) cells are a heterogeneous subset of lymphocytes that provide innate protection against tumor cells and viruses such as the Epstein-Barr virus (EBV). Differentiated NK cells can induce cytotoxicity of target cells through a variety of activating and inhibitory receptors or by employing the activating CD16 (FcgR-IIIA) that can bind to the Fc portion of cell-bound IgG and trigger antibody-dependent cellular cytotoxicity (ADCC). EBV is associated with the development of B cell tumors such as Hodgkin lymphoma (HL) and is present in the malignant Reed-Steinberg (RS) cell of EBV-positive HL. Since NK cells have been shown to contribute to the immune control of EBV-associated B cell tumors in a humanized mouse model, we postulate that patients with EBV-positive HL exhibit a quantitative or qualitative deficiency in NK cell immune control compared to their EBV-negative counterparts.
Patients with newly diagnosed HL referred to the Department of Hematology from Gustave Roussy were included in the study. Baseline characteristics and outcome of the patients were prospectively

recorded. Blood was drawn at diagnosis and for some again 6 months after the end of treatment. We characterized the NK cell phenotype using flow cytometry analysis of peripheral blood and compared the results according to the EBV-status of the HL. NK cell function was assessed by flow cytometry using established protocols for degranulation (natural cytotoxicity) and ADCC (using anti-CD20 Rituximab) against the HLA class I negative EBV-positive LCL721.221 cell line.
36 patients with HL were included in the study between October 2015 and February 2018, among them 26 patients with EBV-negative (72 %) and 10 patients with EBV-positive HL (27 %). We confirmed previous population-based data on HL in developed countries showing an overrepresentation of males (80 % vs. 38 %, P= 0.02) as well as an increased median age (47 vs. 29 years, P= 0.02) in EBV-positive HL compared to EBV-negative HL patients. We found that the late-differentiated $CD56^{dim}CD16+$ NK cell subset was decreased in frequency (median 31 vs. 56 % of total NK cell, P = 0.01) and count (median 18 vs. 47 cells/ul, P = 0.04) at diagnosis in EBV-positive compared to EBV-negative HL patients. Importantly, the decreased frequency of $CD56^{dim}CD16+$ NK cells in EBV-positive HL patients persisted after chemotherapeutic treatment (4 patients studied) suggesting an intrinsic NK cell impairment in these individuals. This decrease was linked to an impaired ADCC against an EBV-infected B cell line in EBV-positive HL compared to their EBV-negative counterparts (median 24 vs. 42 % of $CD107a+CD56^{dim}$ NK cells). Finally, we confirmed previous data showing that EBV-positive HL patients exhibited elevated EBV DNA levels in plasma compared to EBV-negative HL patients (647 vs. 0 EBV DNA copies/ml plasma, P = 0.0017).
Here, we showed that EBV-positive HL patients exhibit decreased frequencies of the ADCC-associated NK cell subset at diagnosis and after treatment. This was linked to an impaired ADCC against an EBV-positive B cell line. This suggests that this particular NK cell dysfunction might lead to inefficient killing of EBV replicating cells and EBV-infected RS cells. Both mechanisms might be linked to the development EBV-positive HL in predisposed individuals. Therefore, enhancing ADCC might represent a novel immune-based therapy in EBV-positive HL.

159

Effect of chronic jetlag induced circadian disruption in breast cancer progression

E. Hadadi (1), A. Bennaceur-Griscelli (2), H. Acloque (1)

(1) Inserm, UMRS935 ESTeam Malignant and Therapeutic Stem Cell Models, Villejuif, France, (2) Service d'hématologie, APHP, GHU Paris Sud, Univ. P. Sud, France

Introduction: Breast cancer is the most common type and one of the major causes of cancer death in woman worldwide. Metastases are responsible for the majority (90%) of mortality from breast cancer and the overall survival rate for metastasised breast cancer is only about 16%. Epidemiology studies linked night shift work with increased risk of cancer suggesting that circadian disruption may interfere with carcinogenesis. Indeed circadian molecular clock has been found to regulate key mechanisms of metabolism, cell migration, immune responses, proliferation and cell survival and recent human and mouse studies linked molecular clock defects, stemness, epithelial-to-mesenchymal transition (EMT) and tumour progression.
Yet, circadian disruption and the underlying mechanisms of its effect on tumour progression in breast cancer are poorly understood and investigated.

Objective: We aim to shed light on the effect of chronic jetlag in mammary tumour development and dissect novel underlying mechanisms regulated by circadian rhythm. Therefore, we studied tumour progression in MMTV-PyMT::MMTV-Luc2 mice with or without chronic jetlag.

Methods: MMTV-PyMT::MMTV-Luc2 transgenic mice were exposed to normal or chronic jetlag conditions for 10 weeks. Tumour progression was followed by in vivo bioluminescence imaging (IVIS). At sacrifice tumours, lung, blood and bone marrow were collected for further analysis using flow cytometry, RT-qPCR, blood analyser and immunostaining.

Results: Circadian disruption significantly increased tumour cell dissemination and metastatic incidence. We observed enhanced stemness of tumour cells and enrichment of Myeloid Derived Suppressor Cells (MDSC) in mice exposed to chronic jetlag. Identification of the key mechanistic regulators is in progress.

Conclusion: Our data suggest that the disturbance of circadian rhythm promote metastatic dissemination through modulation of tumour microenvironment.

160

Cytosolic pH regulates cell cycle progression and tumorigenesis by promoting expression of Cyclin D1

L. M. Koch (1), E. Birkeland (1), C. Chen (1), S. Battaglioni1, M. Meerang (2), M. Kirschner (2), A.J. Ibáñez (3), I. Schmitt-Opitz (2), R. Dechant (1)

(1) Institute of Biochemistry, ETH Zurich, Switzerland, (2) Department of Thoracic Surgery, University Hospital Zurich, Switzerland, (3) Max Plank research group, Pontificial University of Lima, Peru

Faithful regulation of cell growth and division requires the integration of various cellular signals including nutrient and growth factor availability. Although signaling pathways governing cell growth and proliferation are well characterized, molecular mechanisms how different extracellular cues are integrated are less understood, but may involve common second messengers that respond to several extracellular cues. Interestingly, in yeast cytosolic pH has emerged as a cellular determinant regulating cell growth and division, at least in part, through activating TORC1 activity in response to glucose availability. Similarly, stimulation of cell proliferation with growth factors requires an increase of cytosolic pH and increased cytosolic pH has been identified as a hallmark of cancer cells. Thus, cytosolic pH may be a conserved cellular signal regulating cell growth, capable of integrating various extracellular cues, and may contribute to tumorigenesis. Yet, the underlying mechanisms of this regulation have yet to be determined.

Here, we demonstrate that cytosolic pH is regulated by glucose and growth factors in mammalian cells. Increased cytosolic pH in response to growth factor stimulation depends on the Sodium-Hydrogen-Exchanger 1 (NHE1) and Akt, which stimulates NHE1 activity, likely by direct phosphorylation. High cytosolic pH is required during early G1 to promote cell cycle progression. Inactivation of NHE1 arrests cells in early G1 with low Cdk4/Cyclin D1 activity. Further analysis revealed that high cytosolic pH promotes Cyclin D1 transcription and/or regulation of Cyclin D1 mRNA stability. Thus, these data identify a novel molecular mechanism how cytosolic pH regulates cell cycle progression and explain how nutritional and hormonal cues can be integrated to promote cellular proliferation.

As activation of Cdk4/Cyclin D1 activity is frequently associated with the development of cancer, we hypothesized that increased cytosolic pH may promote tumorigenesis by upregulation of Cyclin D1 expression. Indeed, comparing different cell lines derived from malignant pleural mesotheliomas, a cancer characterized by hyperactivated Cyclin D1/Cdk4 activity, high cytosolic pH correlates with Cyclin D1 expression and loss of epithelial morphology. Interestingly, cell lines with high cytosolic pH are hypersensitive to inhibition of NHE1 as well as the Cdk4 inhibitor Ribociclib, suggesting that increased cytosolic pH – mediated by increased NHE1 activity – is critical for the cellular transformation in this cell type by stimulation of Cyclin D1 expression. Thus, we suggest a novel mechanism for how increased cytosolic pH promotes cellular transformation and propose a novel axis of intervention that may be generally applicable for Cyclin D1 dependent tumors.

161

Histological subtyping of Non-Small Cell Lung Cancer by deep learning

R. Casanova (1), E. Murina (2), M. Haberecker (1), H. Honcharova-Biletska (1), B. Vrugt (1), O. Dürr (2), B. Sick (2), A. Soltermann (1)

(1) Institute of Pathology and Molecular Pathology, University Hospital Zurich, Switzerland, (2) Zurich University of Applied Sciences, Winterthur, Switzerland

Background: Non-small cell lung cancer (NSCLC) encompasses a heterogeneous group of histological subtypes. Whereas well-differentiated NSCLC are typically distinguishable by distinctive histologic features such the formation of glandular structures for ADC and the presence of keratin and/or intercellular desmosomes for LSCC, additional immunohistochemistry is often required for poorly differentiated tumors. The objective of this study was to develop an automated framework to classify NSCLC histologic subtypes by deep learning.

Methods: A pre-trained convolutional neural network (CNN) VGG16 was further developed to differentiate ADC from LSCC, on a cohort consisting of 208 NSCLC patients. Patients cohorts were split into a training set (n=140 patients) and test set (n=68). Histologic slides were prepared from formalin-fixed paraffin embedded tumor blocks, stained by Hematoxylin and Eosin. Slides were scanned and 50 frames (128x128 pixels) were prepared for each tumor with a spatial resolution of 2.3µm/pixel.

Results: The performances of the CNN were evaluated on the test set and were compared with the results of three experienced pathologists. In total 66/68 patients were correctly classified by the CNN. Low prediction scores in ADC were significantly associated with the solid ADC subtype. Moreover, additional histologic subgroups could be identified based on morphologic similarity without relying on pathologists labels.
Conclusion: In conclusion, AI supported tissue analysis can provide novel and quantitative classification readouts that could complement routine diagnostic tasks in pathology in the future.

162

The role of NHE1 in regulating intracellular pH and cell growth in pancreatic ductal adenocarcinoma

E. Birkeland, X. Chen, L. Koch, R. Dechant

Institute of Biochemistry, ETH Zurich, Zurich, Switzerland

Increased intracellular pH (pHi) is a hallmark for cell division and tumorigenesis. The Na+-H+ exchanger 1 (NHE1) extrudes cytosolic H+ into extracellular space and is one of the main regulators of pHi in human cells. Interestingly, NHE1 activity is necessary for growth factor-induced increase of pHi and may thus contribute to the regulation of cell proliferation. Indeed, our unpublished evidence strongly suggests that NHE1 activity is required for full mTORC1 activation and Cyclin D1 expression and, consequently, cell cycle progression in human retinal pigment epithelial (RPE) cells. Detailed analysis of NHE1 regulation will be critical to comprehensively understand the regulation of cell growth in health and disease. Our preliminary findings indicate that NHE1 is essential for pH regulation and cell growth in both normal and cancer cell lines.
NHE1 is phosphorylated by Akt at S648 in vitro, suggesting that this phosphorylation may play an important role in regulating NHE1 activity or localization. Previous studies have indicated that Akt activity is necessary for NHE1 localization to the leading edge of the cell membrane during migration. We have observed that NHE1 non-phosphorylatable S648A mutant localizes to the cell membrane similarly to NHE1 WT in human A431 skin cancer cells. Additionally, we found that expression of NHE1 S648A leads to reduced proliferation in A431 compared to A431 expressing NHE1 WT. These findings indicate that Akt phosphorylation of NHE1 is necessary for cell growth and proliferation.
Additionally, NHE1 has been indicated to play a role in pancreatic ductal adenocarcinoma (PDAC) growth and invasion. PDAC is one of the leading cancers in terms of mortality rate, which is generally attributed to late stage diagnosis and limited treatment options. Over 95% of PDACs harbor a mutationally activated form of KRas, which is a main driver of PDAC growth and maintenance. Our preliminary findings shows that NHE1 inhibition reduces pHi in PDAC cells. Additionally, inhibition of Akt, but not MEK, causes a decrease in pHi in PDAC cell lines. These findings indicate a role of NHE1 and Akt activity in regulating PDAC pHi and thus potentially PDAC cell growth, possibly through Akt phosphorylation of NHE1. We aim to further study the role of NHE1 in regulating PDAC growth using in vivo and in vivo-like models such as PDAC organoids. We hope our findings could lead to new therapeutic strategies against PDAC.

163

Gut microbiota modulate T cell trafficking into human colorectal cancer

E. Cremonesi (1), V. Governa (1), J.F. Glaus Garzon (2), V. Mele (1), F. Amicarella (1), M. G. Muraro (1), E. Trella (1), V. Galati-Fournier (3), D. Oertli (4), S. Däster (4), R. A. Droeser (1), B. Weixler (1), M. Bolli (5), R. Rosso (6), U. Nistche (7), N. Khanna (8), A. Egli (9), S. Keck (3), J. Slotta-Huspenina (10), L. M. Terracciano (11), P. Zajac (1), G. C. Spagnoli (1), S. Eppenberger-Castori (11), K. P. Janssen (7), L. Borsig (2), G. Lezzi (12)

(1) Department of Biomedicine, University Hospital of Basel, University of Basel, Basel, (2) Institute of Physiology, University of Zurich, Switzerland, (3) Pediatric Surgery, University Children's Hospital, University of Basel, (4) Department of Surgery, University Hospital Basel, Switzerland, (5) Department of Surgery, St. Claraspital, Basel, (6) Department of Surgery, Ente Ospedaliero Cantonale, Lugano, Switzerland, (7) Department of Surgery, Klinikum Rechts Der Isar, Technical University of Munich, Munich, Germany, (8) Infection Biology, Department of Biomedicine, University of Basel, (9) Applied Microbiology Research, Department of Biomedicine, University of Basel, (10) Institute of Pathology, Technical University of Munich, Munich, Germany, (11) Institute of Pathology, University of Basel, (12) Department of Biomedicine, University of Basel and Department of Surgery, Ente Ospedaliero Cantonale, Lugano

Objective: Tumor infiltrating lymphocytes (TILs) favor survival in human colorectal cancer (CRC). Chemotactic factors underlying their

recruitment remain undefined. We investigated chemokines attracting T cells into human CRCs, their cellular sources and micro-environmental triggers.

Design: Expression of genes encoding immune cell markers, chemokines, and bacterial 16S ribosomal RNA (16SrRNA) was assessed by quantitative RT-PCR in fresh CRC samples and corresponding tumor-free tissues. Chemokine receptor expression on TILs was evaluated by flow cytometry on cell suspensions from digested tissues. Chemokine production by CRC cells was evaluated in vitro and in vivo, upon generation of intraperitoneal or intracecal tumor xenografts in immune-deficient mice. T cell trafficking was assessed upon adoptive transfer of human TILs into tumor-bearing mice. Gut flora composition was analyzed by 16srRNA sequencing.

Results: CRC infiltration by distinct T cell subsets was associated with defined chemokine gene signatures, including CCL5, CXCL9, and CXCL10 for cytotoxic T lymphocytes and T-helper (Th)1 cells, CCL17, CCL22, and CXCL12 for Th1 and regulatory T cells, CXCL13 for follicular Th cells, and CCL20 and CCL17 for IL-17-producing Th cells. These chemokines were expressed by tumor cells upon exposure to gut bacteria in vitro and in vivo. Their expression was significantly higher in intracecal than in intraperitoneal xenografts and was dramatically reduced by antibiotic treatment of tumor-bearing mice. In clinical samples, abundance of defined bacteria correlated with high chemokine expression, enhanced T cell infiltration and improved survival.

Conclusions: Gut microbiota stimulate chemokine production by CRC cells, thus favoring recruitment of beneficial T cells into tumor tissues.

164

Modulatory activities of the extracellular matrix on breast cancer stem cells

F. Fico, A. Santamaria

Department of Oncology, Microbiology & Immunology, University of Fribourg, Fribourg, Switzerland

Metastasis is a major cause of cancer deaths. It is a multistep process composed of several rate-limiting steps that culminates in the colonization and the eventual destruction of secondary organs by tumor cells. Researchers have shown that a subset of cancer cells with stem cell properties, the so-called cancer stem cells (CSCs), are responsible for both sustaining primary tumor growth and leading metastatic colonization. Our lab has recently revealed an essential role of the matricellular protein Transforming Growth Factor b induced (TGFBI) in controlling CSCs and the tumor microenvironment. Human data mining in a cohort of breast cancer patients showed that higher expression of TGFBI correlated with poor prognosis, including shorter distant-metastasis free survival. Accordingly, in MMTV-PyMT mammary gland tumors, deletion of TGFBI lead to a dramatic decrease in metastasis to the lungs. Likewise, limiting dilution assays suggested that TGFBI$^{-/-}$ tumors had a significant reduction in their capacity to reinitiate tumors upon transplantation. Since our analyses indicated that TGFBI was highly expressed by CSCs and monocytes, we analyzed the tumor microenvironment and found that TGFBI affects tumor associated macrophage infiltration and polarization. The lack of TGFBI resulted in tumor vessel normalization, with improved perfusion and decreased hypoxia, suggesting that TGFBI is a key factor promoting aberrant angiogenesis. Overall, these data reveal a novel biological mechanism controlling metastasis that could potentially be exploited to treat breast cancer from a CSC perspective.

165

Integrative RNAseq and Target panel sequencing reveals common and distinct innate and adaptive resistance mechanisms to BRAF inhibitors

P. F. Cheng (1), S. N. Freiberger (2), C. Panetti (1), A. Irmisch (1), R. Dummer (1), M.P. Levesque (1)

(1) Dermatology Clinic, University Hospital Zurich, Switzerland, (2) Pathology, University Hospital Zurich

BRAF inhibitors have been a great success for patients with a BRAF V600 mutation, however, only about 50 % of patients respond to single BRAF inhibitor therapy and the majority of these patients eventually relapse. We have established 53 melanoma cell cultures from biopsies naïve to BRAF inhibitor and progressive on BRAF inhibitor. We in vitro tested each cell line for resistance to BRAF inhibitor and found 27 to be resistant. Surprisingly, a few melanoma cultures from patients who have never been exposed to BRAF inhibitors had innate resistance, while the majority of cell cultures from progressive patients were resistant to in vitro BRAF inhibition. To elucidate the possible resistance mechanisms, we performed RNAseq and targeted panel sequencing on all 53 cultures and found specific subgroups of

gene expression and mutations that define the innate and adaptive resistance populations. One surprising finding was that the phenotype switching signature was one of the resistant mechanisms in common for innate and adaptive resistance, suggesting a selection process for resistant cells during therapy. We also found mechanisms of resistance specific for adaptive resistance, there were many samples that gain mutations in NRAS or acquired a splicing event in BRAF that was not seen in the untreated tumor suggesting a de novo mechanism to resistance. Overall, we noticed several mechanisms to innate and adaptive resistance which highlights tumor and patient heterogeneity when treated with BRAF inhibitors and reinforces the concept for precision medicine in the treatment of melanoma.

166

Abnormalities in microRNA expression profile can contribute to doxorubicin resistance of breast cancer cells

V. Halytskiy

Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine

Background: Doxorubicin is an anthracycline antibiotic that acts as a DNA intercalating agent, inhibiting the topoisomerase II and inducing the apoptotic cell death, mainly due to the accumulation of double-strand DNA breaks (DSB). Doxorubicin resistance is caused by multiple mechanisms, esp. drug efflux, DNA repair, apoptosis prevention and altered (often reduced) level of topoisomerase II-alpha. This research aims to identify in what way the abnormalities in microRNA (miRNA) expression profile in breast cancer cells can confer them the primary and acquired resistance to doxorubicin.

Methods: MiRNA targets within gene transcripts were predicted in silico using the TargetScan software.

Results: Binding sites for miRNAs miR-21, miR-96, miR-183 and miR-365, which are usually upregulated in breast cancer cells, were revealed in transcript of TOP2A gene encoding topoisomerase II-alpha. Transcripts of proapoptotic genes BID, BCL2L11 (BIM), BMF, BAX, BAK1, PMAIP1 (NOXA), and BBC3 (PUMA) as well as transcripts of tumor suppressor genes TP53 and PTEN carry targets for at least one of hyperexpressed miRNAs miR-19, miR-21, miR-23, miR-27, miR-29, miR-155, miR-181, miR-221/222 and miR-375. Downregulation of anti-onco-miRNAs let-7, miR-22, miR-34, miR-101, miR-125, miR-140, miR-143, miR-199, miR-200, miR-203, miR-204 and miR-205 allows overexpression of antiapoptotic genes BCL2, BCL2L1 (Bcl-XL), MCL1 and AKT1 which transcripts carry targets of these miRNAs. In the same way, downregulation of the anti-onco-miRNAs can lead to overexpression of ABCA1/4/12, ABCB1 (MDR1), ABCB6, ABCC1 (MRP1) and ABCC3/5/8/11 genes encoding the ATP binding cassette (ABC) transporters that are responsible for effective efflux of anticancer agents and mediate multiple drug resistance. Moreover, multiple targets for the both up- and downregulated miRNAs were found in transcripts of XRCC5/6, PRKDC, LIG4, DCLRE1C, NHEJ1 (XLF), RAD50/51/51B/51D/52/54B/54L, MRE11A, NBN (NBS1), GEN1, ATM and ATR genes encoding the key elements of non-homologous end joining and homologous recombination pathways, responsible for DSB repair.

Conclusions: Primary insensitivity to doxorubicin can be caused by tumor-associated shifts in miRNA expression, which contribute to increased Bcl-2/Akt survival signaling, to overexpression of genes responsible for DSB repair and drug efflux, to silencing of topoisomerase II-alpha and tumor suppressor genes. Acquired resistance can be simple achieved with further tuning of the miRNA levels. Probably, equilibrium of above down- and up-regulated miRNAs may predict the resistance or susceptibility of breast cancer cells to doxorubicin.

1. Halytskiy V. Multiple drug resistance in breast cancer cells: miRNAome dysregulation can facilitate expression of genes encoding the ATP binding cassette (ABC) transporters. The Breast, 2017, 36 (Suppl. 1): S57.

2. Halytskiy V. Shifts in miRNAome underlie anoikis resistance of breast cancer cells. The Breast, 2017, 32 (Suppl. 1): S33.

3. Halytskiy V. Shifts in miRNA expression affect DNA repair mechanisms in breast cancer cells. The Breast, 2015, 24 (Suppl. 1): S26-S27.

167

Increased sensitivity to apoptosis upon endoplasmic reticulum stress-induced activation of the unfolded protein response in chemotherapy-resistant malignant pleural mesothelioma

D. Xu (2), H. Yang (3), Z. Yang (3), R. Peng (1), S. Ralph (1)

(1) Division of General Thoracic Surgery, Inselspital, Bern University Hospital, Bern, Switzerland, (2) Department for BioMedical Research,

University of Bern, Bern, (3) Graduate School for Cellular and Biomedical Sciences, University of Bern

Background: Standard treatment for advanced malignant pleural mesothelioma (MPM) is a cisplatin/pemetrexed (MTA) regimen; however, this is confronted by drug resistance. Proteotoxic stress in the endoplasmic reticulum (ER) is a hallmark of cancer and some rely on this stress signalling in response to cytotoxic chemotherapeutics. We hypothesise that ER stress and the adaptive unfolded protein response (UPR) play a role in chemotherapy resistance of MPM.

Methods: In vitro three-dimensional (3D) and ex vivo organotypic culture were used to enrich a chemotherapy-resistant population and recapitulate an in vivo MPM microenvironment, respectively. Markers of ER stress, the UPR and apoptosis were assessed at mRNA and protein levels. Cell viability was determined based on acid phosphatase activity.

Results: MPM cells with de novo and/or acquired chemotherapy resistance displayed low ER stress, which rendered the cells hypersensitive to agents that induce ER stress and alter the UPR. Bortezomib, an FDA-approved proteasome inhibitor, selectively impairs chemotherapy-resistant MPM cells by activating the PERK/eIF2α/ATF4-mediated UPR and augmenting apoptosis.

Conclusions: We provide the first evidence for ER stress and the adaptive UPR signalling in chemotherapy resistance of MPM, which suggests that perturbation of the UPR by altering ER stress is a novel strategy to treat chemotherapy-refractory MPM.

168

Generation of a living biobank of patient-derived pancreatic cancer organoids: challenges and potential applications

R. Klar* (1), S. Hussung* (1, 5), A. Devisme (2), G. Andrieux (2, 4, 5), S. Michalczyk (1), K. Fritsch (1), M. Boerries* (2, 4, 5), U. Wittel* (3),R. Fritsch* (1, 5, 6)
* contributed equally

(1) Department of Medicine I (Hematology, Oncology and Stem Cell Transplantation), Freiburg, Germany, (2) Institute of Molecular Medicine and Cell Research, Freiburg, Germany, (3) Department of Surgery, Medical Center University of Freiburg, Germany, (4) German Cancer Research Center (DKFZ), Heidelberg, Germany, (5) German Cancer Consortium (DKTK), partner site Freiburg, German Cancer Research Center (DKFZ), Heidelberg, Germany, (6) Department of Medical Oncology and Hematology, Zurich University Hospital, Zurich, Switzerland

The clinical management of pancreatic adenocarcinoma has remained extremely challenging. Pre-clinical advances and the extensive use of comprehensive molecular analysis have profoundly improved our understanding of the disease. With a few notable exceptions, however, translational research efforts have failed to establish exploitable genotype-phenotype relationships, and only very little progress has translated into the clinic. Patient-derived pancreatic cancer organoids (PDOs) preserve genotype and phenotype of individual tumors and can be studied ex vivo in a co-clinical manner. To what extent and under which conditions these cultures can predict clinical course or uncover individual therapeutic vulnerabilities remains to be fully established.
For this project, we set out to generate a large living biobank of PDOs derived from patients undergoing resection or surgical biopsy of pancreatic adenocarcinoma at the University of Freiburg Pancreatic Cancer Center. Following informed consent, organoid cultures of hitherto 70 patients have been generated. Organoid cultures undergo a standardized work-up including histology and quantitative KRAS mutational analysis by digital droplet PCR (ddPCR), followed by NGS panel or whole exome sequencing. Initial organoid generation success rate across all samples was 83% (58/70 pts.). However, a significant subset of cultures ceased to grow within 5-10 passages ex vivo, lowering overall efficiency to 53% (37/70 pts.). Failure to generate tumor organoids was associated with low tumor cell content in the biopsy material and subsequent outgrowth of non-tumor "wild type" organoids during early passaging. For successfully established organoids, we are optimizing a co-clinical work-flow assessing the ex vivo invasive and in vivo metastatic capacity of individual PDOs, correlating functional results with comprehensive molecular profiling and clinical data. Mechanistic studies aim to identify and validate key drivers of systemic dissemination in PDOs. With a future clinical trial in mind, we also have been establishing an ex vivo drug screening protocol aimed at the identification of individual drug vulnerabilities that could be exploited therapeutically.
In summary, we report the generation and ongoing analysis of a large collection of patient-derived pancreatic cancer organoids, discussing challenges and potential applications.

Charles Rodolphe Brupbacher Stiftung

Charles Rodolphe Brupbacher Foundation

Mme Frédérique Brupbacher
Portrait by Peter Cerutti

Charles Rodolphe Brupbacher Stiftung

Die Stiftung hat das Ziel, die Krebsforschung in der Schweiz und international zu fördern.

Wichtigstes Element ihrer Tätigkeit ist die Verleihung des Charles Rodolphe Brupbacher Preises für Krebsforschung, verbunden mit einem wissenschaftlichen Symposium in Zürich.

Die Stifterin

Frau Frédérique Brupbacher hat im November 1991 in Verehrung ihres Gatten, Charles Rodolphe Brupbacher, eine Stiftung mit Sitz in Vaduz errichtet. Die Stiftung verleiht alle zwei Jahre den Charles Rodolphe Brupbacher Preis für Krebsforschung an Wissenschaftler, die in der Grundlagenforschung herausragende Leistungen erbracht haben. Die Preisverleihung findet statt im Rahmen eines internationalen wissenschaftlichen Symposiums.

Auf Antrag der Medizinischen Fakultät ernannte die Universitätsleitung Frau Frédérique Brupbacher 2005 zum Ständigen Ehrengast der Universität Zürich, in Anerkennung der grossen Verdienste, die sie sich mit ihrem Altruismus und ihrem Engagement für die Krebsforschung erworben hat. Durch ihre Initiative und ihren persönlichen Einsatz konnte die Krebsforschung im Raum Zürich nachhaltig gestärkt werden. Am 20. Juni 2001 ernannte Präsident Jacques Chirac sie zum Chevalier de la Légion d'Honneur.

Charles Rodolphe Brupbacher Foundation

The mission of the Foundation is to foster cancer research in Switzerland and internationally.

The key element of its activities is the Charles Rodolphe Brupbacher Prize for Cancer Research which is awarded in association with a scientific symposium in Zurich.

The Founder

In honour of her late husband Charles Rodolphe Brupbacher, Mrs. Frédérique Brupbacher set up a foundation registered in Vaduz, Liechtenstein, in November 1991. The Foundation's mission is to present the biennial Charles Rodolphe Brupbacher Prize for Cancer Research to a scientist with internationally acknowledged meritorious achievements in the field of fundamental research. The Prize is awarded in the context of a scientific symposium.

The Executive Board of the University of Zurich appointed Mrs. Frédérique Brupbacher in 2005 as a permanent Guest of Honor of the University, in appreciation of her altruism and her engagement for cancer research. Through her personal committment, cancer research in Zurich has been significantly strengthened. President Jacques Chirac of France appointed her to Chevalier de la Légion d'Honneur.

Charles Rodolphe Brupbacher

1909 – 1987

Charles Rodolphe Brupbacher wurde am 5. Februar 1909 in Zürich als Bürger von Wädenswil geboren. Sein Vater, C.J. Brupbacher, war Inhaber einer Privatbank am Paradeplatz. Die Mutter, geborene Französin, legte grossen Wert auf eine zweisprachige Erziehung des Sohnes. Dies erklärt auch seine lebenslange, enge Beziehung zu Frankreich, zu dessen Geschichte und Kultur und seine dauernde, grosszügige Unterstützung der Ecole française und der Alliance française in Zürich. Sein jahrzehntelanger Einsatz für die Anliegen der französischen Kultur wurde mehrfach durch die jeweiligen Staatspräsidenten geehrt:

1961 Präsident Charles De Gaulle
Ernennung zum Chevalier de la Legion d'Honneur
1973 Präsident Georges Pompidou
Ernennung zum Officier de la Legion d'Honneur
1979 Präsident Valéry Giscard d'Estaing
Ernennung zum Commandeur de l'Ordre National de Merite

Schon früh zeigte sich bei Charles Rodolphe Brupbacher eine ausgesprochene Sprachbegabung; er beherrschte fünf Sprachen fliessend. Als musikalisches Wunderkind mit dem absoluten Gehör widmete er sich der Interpretation klassischer Musik und bedauerte zeitlebens, dass er auf eine Ausbildung als Konzertpianist verzichten musste. Charles Rodolphe Brupbacher besuchte die Schulen in Zürich und Paris.

Charles Rodolphe Brupbacher was born on February 5, 1909 in Zurich, as a citizen of Wädenswil. His father, C.J. Brupbacher, owned a private bank on Paradeplatz. His mother, a French citizen, placed great importance on a bilingual education for her son. This explains his lifelong, close relationship with France, its history and culture. This is also reflected by his continuous and generous support of the École française and the Alliance française in Zurich. Several French Presidents honoured his commitment to French cultural issues:

1961 President Charles De Gaulle
Election to Chevalier de la Legion d'Honneur
1973 President Georges Pompidou
Election to Officier de la Legion d'Honneur
1979 President Valéry Giscard d'Estaing
Election to Commandeur de l'Ordre National de Merite

At an early age, Charles Rodolphe Brupbacher showed a distinct talent for languages, and he spoke five of them fluently. As a musical prodigy with perfect pitch, he devoted himself to the interpretation of classical music. He regretted throughout his life that he had not been able to receive an education as a concert pianist. Charles Rodolphe Brupbacher attended schools in Zurich and Paris.

Mit 18 Jahren musste er auf Verlangen seines Vaters die Ausbildung am Gymnasium in Zürich und Paris aufgeben und eine Banklehre absolvieren. Anschliessend besuchte er ab 1929 immer wieder die Vereinigten Staaten, sowie Lateinamerika und trat so in Beziehung zu grossen Persönlichkeiten in führender Stellung.

Nach seiner Rückkehr in die Schweiz gründete er, als damals jüngster Bankier, mit 24 Jahren die auf Vermögensverwaltung spezialisierte Bank «Affida» am Paradeplatz in Zürich. Sein Erfolg war in hohem Masse seinen Geschäftsprinzipien zu verdanken. Dazu gehörte der Aufbau eines Informationsnetzes, welches ihn mit den wichtigsten finanziellen und politischen Zentren verband. Von grosser Bedeutung waren dabei seine detaillierten Kenntnisse der internationalen Rechtsprechung, der Nationalökonomie und ganz speziell auch von Währungsfragen. Nach 40jähriger Tätigkeit verkaufte er die Affidabank an die Schweizerische Kreditanstalt (Credit Suisse).

Auf Grund seiner umfassenden Kenntnisse wurde Charles Rodolphe Brupbacher 1938 von Prof E. Böhler in die Gruppe für Konjunkturbeobachtung der Eidgenössischen Technischen Hochschule (ETH) berufen. Als deren Mitglied nahm er auch an Besprechungen kriegswirtschaftlicher Probleme in Bern teil.

Als anerkannter Fachmann in Währungsfragen wurde Charles Rodolphe Brupbacher nach dem Kriege als einziger Beobachter aus der Schweiz zu den internationalen Währungskonferenzen eingeladen. Seine persönlichen Beziehungen zu wichtigen Politikern in den USA erlaubten es ihm, durch jahrelange, zähe Verhandlungen grosse schweizerische Guthaben zu deblockieren.

Auch bemühte sich Charles Rodolphe Brupbacher intensiv um die Probleme, welche sich bei dem Wiederaufbau der Montanindustrie zwischen Deutschland und den Alliierten entwickelt hatten. In diesem Zusammenhang wurde er von der französischen Regierung und der Regierung von Nordrhein-Westfalen zur Teilnahme an dem Treffen anlässlich der ersten Reise von General de Gaulle nach Deutschland eingeladen.

Schon im Jahre 1963 hat Charles Rodolphe Brupbacher an der ETH eine Stiftung zur Unterstützung von Studierenden auf dem Gebiet der Sozialwissenschaften gegründet, die seither laufend Stipendien vergibt.

Charles Rodolphe Brupbacher starb am 1. Januar 1987 und hinterliess seine Ehefrau Frédérique, die er 1953 geehelicht hatte.

At the age of 18, however, he had to give up his education at the Gymnasium (College) to undertake a banking apprenticeship. He visited the United States and Latin America in 1929 and frequently thereafter: first, for the purpose of training; later, to keep himself informed.

At the Paradeplatz in Zurich, at the age of only 24, he established the «Affida Bank», which specialized in asset management. His success was largely due to a committment to personal business integrity. His achievements included the setting-up of an information network that connected him with important financial and political centres. His detailed knowledge of international commercial law, of national economics and, especially, of currency policy were great assets. After 40 years, he sold the «Affida Bank» to Credit Suisse.

Based on his detailed knowledge, Charles Rodolphe Brupbacher was invited by Professor E. Böhler in 1938 to join a select group formed at the Swiss Federal Institute of Technology (ETH), which met to monitor the economy. As a member, he often took part in discussions in Bern of wartime economic problems.

As a recognised expert in monetary policy, Charles Rodolphe Brupbacher was the only observer from Switzerland to be invited after the war to the international currency conferences. His personal relationship with prominent politicians in the United States enabled him, through years of negotiations, to release major Swiss assets.

Charles Rodolphe Brupbacher also helped to attenuate problems which had developed between Germany and the Allies regarding the restoration of the coal and steel industry. In this context, he was invited by the Government of France and by the State of North Rhine-Westphalia to participate in the meeting on the occasion of General de Gaulle's first visit to Germany.

Already in 1963, Charles Rodolphe Brupbacher established a Foundation at the ETH with the objective of supporting students in the field of social sciences. Since then, the Foundation has continuously granted scholarships.

Charles Rodolphe Brupbacher died on January 1, 1987, survived by his wife Frédérique whom he married in 1953.

Stiftungsrat

Der Stiftungsrat verwaltet die Stiftung und vertritt sie nach aussen. Er trifft die Entscheide über Preisverleihungen und die begleitenden wissenschaftlichen Symposien.

Mitglieder des Stiftungsrates:

Georg C. Umbricht, LL.M., Zürich

Prof. Dr. Michael O. Hengartner, Zürich

Veit de Maddalena, Zürich

Dr. Lukas S. Keller, Zürich

Prof. Dr. Alexander Knuth, Zürich

Prof. Dr. Holger Moch, Zurich

Dr. Ernst Walch, Vaduz

Foundation Board

The Foundation Board administers the Foundation and determines policy. The Foundation Board makes final decissions regarding Prizes and Symposia.

Members of the Foundation Board:

Georg C. Umbricht, LL.M., Zurich

Prof. Dr. Michael O. Hengartner, Zurich

Veit de Maddalena, Zurich

Dr. Lukas S. Keller, Zurich

Prof. Dr. Alexander Knuth, Zurich

Prof. Dr. Holger Moch, Zurich

Dr. Ernst Walch, Vaduz

Wissenschaftlicher Beirat

Der Wissenschaftliche Beirat nominiert Kandidaten für den Charles Rodolphe Brupbacher Preis für Krebsforschung und empfiehlt Redner für die wissenschaftlichen Symposien.

Mitglieder/Members:

Prof. Dr. Lauri A. Aaltonen
Biomedicum Helsinki
University of Helsinki
Helsinki, Finland

Prof. Dr. Nancy E. Hynes
Friedrich Miescher Institute
for Biomedical Research
Basel, Schweiz

Prof. Dr. Josef Jiricny
Department of Biology
Institute of Biochemistry, ETH
Zurich, Switzerland

Prof. Dr. Markus G. Manz
Department of Hematology and Oncology
University Hospital Zurich
Zurich, Switzerland

Prof. Dr. Sir Alex Markham
Institute of Molecular Medicine
University of Leeds
Leeds, U.K.

Scientific Advisory Board

The Scientific Advisory Board nominates candidates for the Charles Rodlphe Brupbacher Prize for Cancer Research. The Advisory Board also suggests Symposia speakers.

Prof. Dr. Miriam Merad
Hess Center Tisch Cancer Institute
Mount Sinai School of Medicine
New York, USA

Prof. Dr. Holger Moch
University Hospital Zurich
Institute for Clinical Pathology
Zurich, Switzerland

Prof. Dr. Lukas Sommer
University of Zurich
Institute of Anatomy
Zurich, Switzerland

Prof. Dr. med. Rainer Weber
Dean of the Medical Faculty
University of Zurich
Zurich, Switzerland

Ehrenmitglied/Honorary Member

Prof. Dr. med. Dr. h.c. Paul Kleihues
Professor emeritus of Neuropathology
University of Zurich
Zurich, Switzerland

Kontakt

Sekretariat
C. R. Brupbacher Stiftung
Zollstrasse 2
Postfach 1611
9490 Vaduz
Fürstentum Liechtenstein

Wissenschaftliches Sekretariat
C. R. Brupbacher Foundation
Bahnhofstrasse 24
Postfach
8024 Zürich
Schweiz
Tel. +41 44 226 99 60
office@brupbacher-foundation.org
www.brupbacher-foundation.org

Alle Korrespondenzen und Anfragen bezüglich des Symposiums sind an das Wissenschaftliche Sekretariat zu richten.

Contact

Secretariat
C. R. Brupbacher Stiftung
Zollstrasse 2
P.O. Box 1611
9490 Vaduz
Principality of Liechtenstein

Scientific Secretariat
C. R. Brupbacher Foundation
Bahnhofstrasse 24
P.O. Box
8024 Zurich
Switzerland
Ph +41 44 226 99 60
office@brupbacher-foundation.org
www.brupbacher-foundation.org

All correspondence and enquiries regarding the Symposium should be addressed to the Scientific Secretariat.

Address List
Speakers and Board Members

Lauri A. AALTONEN
Genome-Scale Biology Program &
Center of Excellence in Cancer Genetics
Biomedicum Helsinki
P.O. Box 63 (Haartmaninkatu 8)
00014 University of Helsinki
Finland
lauri.aaltonen@helsinki.fi

Nicola ACETO
University of Basel
Department of Biomedicine
Cancer Metastasis Laboratory Mattenstrasse 28
4058 Basel
Nicola.Aceto@unibas.ch

Bernd BODENMILLER
University of Zurich
Institute of Molecular Life Sciences
Winterthurerstrasse 190
8057 Zurich
Switzerland
bernd.bodenmiller@imls.uzh.ch

G. Steven BOVA
University of Tampere
Faculty of Medicine and Life Sciences Arvo
Ylpön katu 34
33014 Tampere
Finland
g.steven.bova(at)uta.fi

Gerhard CHRISTOFORI
University of Basel
Department of Biomedicine
Institute of Biochemistry & Genetics
Mattenstrasse 28
4058 Basel
gerhard.christofori@unibas.ch

Pierre CLOSE
University of Liège
Giga Institute
Avenue de l'Hopital, 11
4000 Liège
Belgique
Pierre.Close(at)ulg.ac.be

Veit DE MADDALENA
Bellevue Vermögensverwaltung
Seestrasse 16
8700 Küsnacht
Switzerland
veit@maddalena.com

Karin DE VISSER
The Netherlands Cancer Institute Division of Tumor Biology and Immunology Oncode Institute
Plesmanlaan 121 / Postbus 90203
1006 CX Amsterdam
The Netherlands
k.d.visser@nki.nl

Paul FRENETTE
Albert Einstein College of Medicine
1301 Morris Park Avenue , Room 101
Bronx, NY 10461
USA
paul.frenette@einstein.yu.edu

Richard J. GILBERTSON
Cambridge Cancer Center
CRUK Cambridge Institute
Robinson Way
Cambridge CB2 0RE
United Kingdom
Richard.Gilbertson@cruk.cam.ac.uk

Michael N. HALL
Biozentrum
University of Basel
Klingelbergstrasse 70
4056 Basel
Switzerland
m.hall@unibas.ch

Peter HILLMEN
University of Leeds
St. James's University Hospital
Leeds Institute of Cancer & Pathology
Beckett Street
Leeds, LS9 7TF
United Kingdom
P.Hillmen@leeds.ac.uk

Michael O. HENGARTNER
University of Zurich
Künstlergasse 15
8001 Zurich
Switzerland
rektor@uzh.ch

Nancy E. HYNES
Friedrich Miescher Institute for Biomedical Research
Maulbeerstrasse 66
P.O. Box 3775
4058 Basel
Switzerland
nancy.hynes@fmi.ch

Josef JIRICNY
ETH Zürich
Department of Biology
Institute of Biochemistry
Otto-Stern-Weg 3
8093 Zurich
Switzerland
jjiricny@ethz.ch

Nada JABADO
Montreal Children's Hospital
McGill University
Research Institute of the McGill University Health Center
Montreal, QC,CANADA
nada.jabado@mcgill.ca

Johanna JOYCE
Ludwig Institute for Cancer Research
University of Lausanne
Chemin des Boveresses 155
Épalinges VD, 1066
Switzerland
johanna@joycelab.org

Lukas S. KELLER
Röntgenstrasse 84
8005 Zürich
Switzerland
lukas_keller@alumni.uzh.ch

Paul KLEIHUES
Pestalozzistrasse 5
8032 Zürich
Switzerland
kleihues@pathol.uzh.ch

Alexander KNUTH
National Center for Cancer Care & Research
NCCCR
Hamad Medical Corporation
P.O. Box 3050
Doha, Qatar
kknuth@hmc.org.qa

Peter LICHTER
German Cancer Research Center (DKFZ) Im Neuenheimer Feld 280
69120 Heidelberg
Germany
peter.lichter@dkfz-heidelberg.de

Markus G. MANZ
Klinik für Hämatologie
UniversitätsSpital Zürich
Rämistrasse 100
8091 Zürich
Switzerland
markus.manz@usz.ch

Sir Alexander F. MARKHAM
School of Medicine
University of Leeds
Wellcome Trust Brenner Building
St James's University Hospital
Leeds LS9 7TF, UK
a.f.markham@leeds.ac.uk

Miriam MERAD
Mount Sinai School of Medicine
Hess Center Tisch Cancer Inst, 5th Fl, Room 118, 1470 Madison Avenue
New York, NY 10029
USA
miriam.merad@mssm.edu

Holger MOCH
Institut für Pathologie und Molekularpathologie
Schmelzbergstrasse 12
8091 Zürich
Switzerland
pathologie@usz.ch

Sean MORRISON
Howard Hughes Medical Institute and Children's Medical Center Research Institute University of Texas Southwestern Medical Center
Dallas, TX 75390, USA
sean.morrison@utsouthwestern.edu

Anne MUELLER
University of Zurich
Institute of Molecular Cancer Research
Winterthurerstrasse 190
8057 Zurich
mueller@imcr.uzh.ch

Serena NIK-ZAINAL
School of Clinical Medicine
University of Cambridge
Addenbrooke's Hospital
Cambridge CB2 0QQ
United Kingdom
sn206@cam.ac.uk

Daniel S. PEEPER
Netherlands Cancer Institute
Room B6.039
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands
d.peeper@nki.nl

Alain PUISIEUX
Cancer Research Center of Lyon
INSERM U1052 - CNRS UMR 5286 University of Lyon, Centre Léon Bérard
28 Rue Laennec
69373 LYON CEDEX 08,
France
alain.puisieux@lyon.unicancer.fr

Antoni RIBAS
UCLA Medical Center Hematology/
Oncology 100 UCLA Medical Plaza
Suite 550
Los Angeles, California 90095
USA
aribas@mednet.ucla.edu

Andrew W. ROBERTS
Cancer and Haematology Division
Walter and Eliza Hall
Institute of Medical Research
Royal Parade
Parkville, VIC 3052
Victoria, Australia,
roberts@wehi.edu.au

Lukas SOMMER
University of Zurich
Institute of Anatomy
Winterthurerstrasse 190
8057 Zurich
lukas.sommer@anatomy.uzh.ch

Jean-Philippe THEURILLAT
Institute of Oncology Research
Oncology Institute of Southern Switzerland
Bellinzona, TI 6500
Switzerland
JeanP_Theurillat@ior.usi.ch

Ian TOMLINSON
Institute of Cancer and Genomic Sciences
University of Birmingham
Edgbaston
Birmingham B15 2TT
United Kingdom
i.tomlinson@bham.ac.uk

Andreas TRUMPP
German Cancer Research Center (DKFZ)
Im Neuenheimer Feld 280
69120 Heidelberg
Germany
a.trumpp@dkfz.de

Georg C. UMBRICHT
Umbricht Rechtsanwälte
Bahnhofstr. 22
Postfach
8024 Zürich
Switzerland
g.umbricht@umbricht.ch

Nicola VALERI
ICR The Institute of Cancer Research
15 Cotswold Road
Sutton, London SM2 5NG
United Kingdom
nicola.valeri@icr.ac.uk

Matthew VANDER HEIDEN
Koch Institute for Integrative Cancer
Research at MIT
77 Massachusetts Ave.
Cambridge MA 02139
USA
mvh@mit.edu

Rainer WEBER
Universität Zürich
Dekanat der Medizinischen Fakultät
Pestalozzistrasse 3/5
8091 Zürich
sekretariat@dekmed.uzh.ch

Ernst J. WALCH
WalPart Trust reg.
Zollstrasse 2 / Postfach 1611
9490 Vaduz
Fürstentum Liechtenstein
mail@walchschurti.net

Index

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z