Charles Rodolphe Brupbacher Stiftung

Breakthroughs in Cancer Research and Therapy

Edited by

Markus G. Manz
Klaus Rajewsky
Paul Kleihues

Ponte Press
2013

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Markus G. Manz, Klaus Rajewky, Paul Kleihues (Eds.)
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Charles Rodolphe Brupbacher Preis für Krebsforschung 2013

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 2013 wird verliehen an:

Michael Karin, San Diego, USA

Charles Rodolphe Brupbacher Prize for Cancer Research 2013

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 Prize ceremony takes place within the framework of a Scientific Symposium, which includes the Charles Rodolphe Brupbacher Public Lecture.

The recipient of the 2013 Prize is:

Michael Karin, San Diego, USA

Charles Rodolphe Brupbacher Preis für Krebsforschung

UniversitätsSpital Zürich, Grosser Hörsaal Ost, Gloriastrasse 29, 8091 Zürich
Tramstop: Platte
Donnerstag, 31. Januar 2013, um 17:00 Uhr

Begrüssung
Prof. Dr. Andreas Fischer, Rektor der Universität Zürich
Prof. Dr. Klaus W. Grätz, Dekan, Präsident des Wissenschaftlichen Beirats

S. Rachmaninov, Prelude Nr. 5 op 23

Laudatio
Prof. Dr. Michael Karin

durch

Prof. Dr. Klaus Rajewsky

F. Chopin, Etüde Nr. 1 op 25

Preisverleihung
Mme. Frédérique Brupbacher, Präsidentin der Stiftung

A. Babadajanyan, Elegia

Referat des Preisträgers
Prof. Dr. Michael Karin

F. Chopin Etüde Nr. 12 op 25 in c moll

Schlussworte
Prof. Dr. Klaus W. Grätz

Apéro

Charles Rodolphe Brupbacher Prize for Cancer Research

University Hospital Zurich, Lecture Hall East, Gloriastrasse 29, 8091 Zurich
Tramstop: Platte
Thursday, January 31st, 2013, 17:00 pm

Welcome
Prof. Dr. Andreas Fischer, Rector of the University of Zurich
Prof. Dr. Klaus W. Grätz, Dean, President of the Scientific Advisory Board

S. Rachmaninov, Prelude Nr. 5 op 23

Laudatio
Prof. Dr. Michael Karin

by

Prof. Dr. Klaus Rajewsky

F. Chopin, Etude Nr. 1 op 25

Award
presented by
Mme. Frédérique Brupbacher, President of the Foundation

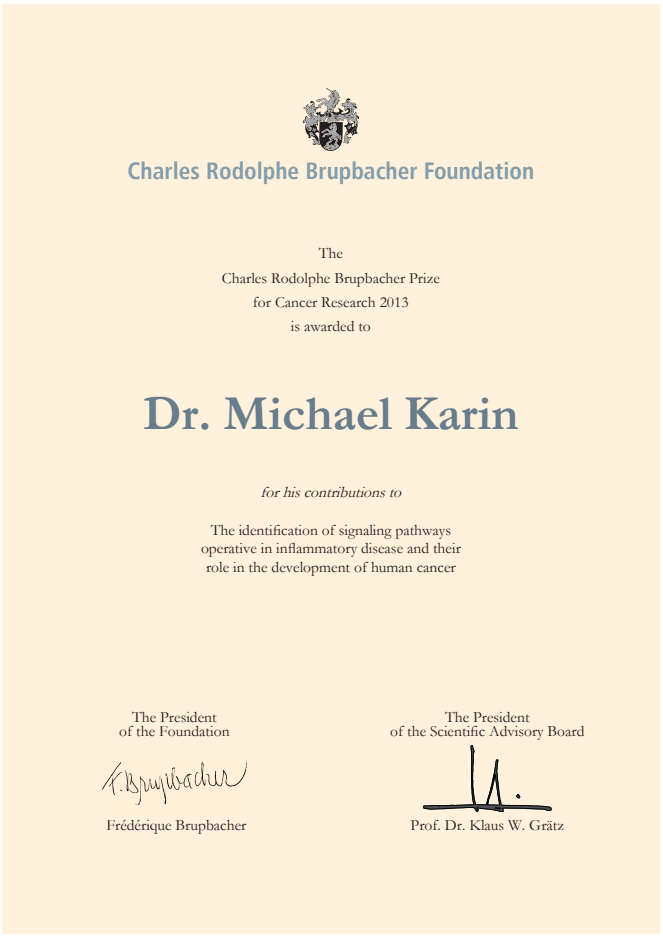
A. Babadajanyan, Elegia

Acceptance Speech
Prof. Dr. Michael Karin

F. Chopin Etude Nr. 12 op 25 in c moll

Final address
Prof. Dr. Klaus W. Grätz

Apéro



Laudatio

Klaus Rajewsky

The notion of a link between cancer and inflammation, famously put forward by Rudolf Virchow in his academic lectures on "die krankhaften Geschwülste" in the winter semester 1862/63 in Berlin, has a long and rich tradition. Inflammation, a hallmark of innate immunity, represents a defense of the organism against tissue damage and pathogen invasion. It involves the death and regeneration of cells and usually resolves within a short time. However, inflammatory processes often develop into a chronic, pathological condition, and it appears plausible that this can be accompanied by a deregulation of cellular proliferation, and, ultimately, malignant growth – "wounds that won't heal".

Only recently, however, with the advent of modern methods of molecular biology and in particular, mouse genetics, has a picture emerged in which we begin to mechanistically understand the complex ways in which chronic inflammatory processes and malignant growth can go hand-in-hand and promote each other. It is in this general context that we celebrate Michael Karin today as a scientific pioneer of the highest caliber.

Michael Karin was born in Israel, studied biology at Tel Aviv University and then moved to the United States to work on his PhD thesis at the University of California at Los Angeles. After two short postdoctoral stays with Beatrice Mintz and John Baxter from 1979 to 1981 he began his independent scientific career, settling in 1986 at the University of California in San Diego, where he is a professor in the Department of Pharmacology since 1989.

His first scientific publication, resulting from his thesis work with Harvey Herschman, was a paper in Science on the induction of metallothionein synthesis in HeLa cells by dexamethasone. Control of gene expression by external stimuli through cellular receptors, intracellular signaling cascades and transcription factors & transcriptional control became his obsession, combining methods of biochemistry, molecular biology and imaginatively, as they became available, mouse genetics. This led him from the analysis of basic molecular mechanisms to the study of pathophysiology in animals. In an amazing, steady stream of publications in top scientific journals to this day he and his colleagues have contributed a truly overwhelming wealth of discoveries and new insights relating to diverse biological processes, ranging from the role of protein phosphorylation in transcriptional control and basic principles of signal transduction to signaling cascades involved in cellular stress, innate immunity, inflammation and cancer. This included the discovery or in-depth characterization of key protein kinases, namely Jun kinase (JNK) that controls stress responses, and IκB kinase (IKK), a protein complex with a central role in the activation of the NF-κB pathway. This latter pathway is the main player in the control of inflammatory processes and innate immunity, and it is here that genetically tailored mouse models provided direct mechanistic links between inflammation and cancer.

Two papers published in 2004, one from Michael Karin's group and the other from Yinon Ben-Neriah and colleagues, described mouse models of two classical inflammation-associated human malignancies, colitis-associated colon cancer on the one hand, and hepatocellular carcinoma on the other. In both cases NF-κB activity was critical for cancer pathogenesis, amazingly in both the cancer cells themselves and

inflammatory cells in the cancer environment. In this complex scenario, anti-apoptotic proteins encoded by NF-κB target genes in the tumor cells cooperate with NF-κB controlled cytokines produced by neighboring myeloid and endothelial cells and promoting tumor cell growth and survival. One of these factors is interleukin 6, which Karin and colleagues demonstrated to play a role in both colon cancer and hepatocellular carcinoma, contributing in the latter case to the preferential occurrence of this malignancy in males, as also observed in the human. Additional complexity of this cellular interplay comes from the requirement for NF-κB activity at distinct phases of tumor development, and distinct functional activities of NF-κB transcription factors in different cell types and cellular contexts. In the past few years, the Karin laboratory has uncovered fascinating facets of these matters. To name just a few, NF-κB driven lymphotoxin production in B lymphocytes is required for the development of castration-resistant prostate cancer, through upregulation of IKKα in the tumor cells; and IKKα is also required for prostate cancer metastasis. Karin and colleagues have also provided compelling evidence that obesity predisposes for hepatocellular carcinoma through chronic inflammation, caused by fat deposition in the liver and resulting in the overproduction of cytokines like interleukin 6 and tumor necrosis factor.

I will just mention in passing that all of the above work is clearly of utmost clinical relevance and indispensable for the development of new therapies in this general area, and close by saying that Michael Karin is a true scientific giant in the biomedical field, who took rigorous basic research right into the realm of human disease, in particular the cancer field. He did this by an insatiable curiosity, energy and ambition, none of which he has lost even a bit over the years. My own first contact with him taught me all about scientific competition; while it fortunately ended in a draw, my lesson was to better not compete with him. He is a master of generating new ideas and concepts by bringing together elements from diverse fields, based on an enzyclopedic knowledge of the literature. No greater fun than to discuss science (or, for that matter, other matters) with him, and to have him as a participant in a scientific meeting. Congratulations, Michael, for this highly deserved honor!

Michael Karin

Summary Curicculum vitae

Appointment University of San Diego, Calefornia

Address Department of Pharmacology
La Jolla, California 92093-0723

Date of Birth May 25, 1951

Education Tel Aviv University, Tel Aviv, Israel
B.Sc. Biology, 1975
University of California, Los Angeles
Ph.D. Molecular Biology, 1979

Professional Appointments

1975 – 1979	Graduate Student, Molecular Biology Institute, UCLA. Supervisor: Dr. Harvey Herschman
1979 – 1980	Postdoctoral Fellow, Fox Chase Institute for Cancer Research, Philadelphia. Supervisor: Dr. Beatrice Mintz
1980 – 1981	Postdoctoral Fellow, Department of Medicine and Biochemistry, University of California, San Francisco. Supervisor: Dr. John Baxter

1981 – 1982	Assistant Research Biochemist, Metabolic Research Unit, University of California, San Francisco
1982 – 1985	Assistant Professor of Microbiology, School of Medicine, University of Southern California
1986 –1987	Associate Professor, Department of Medicine, University of California, San Diego
1987 – 1989	Associate Professor, Department of Pharma cology, University of California, San Diego
1989 – Present	Professor, Department of Pharmacology, University of California, San Diego
1993 – 2005	Founder and Consultant, Signal Pharmaceuticals (currently Celgene Pharma ceuticals), San Diego, CA

Editorial Boards

DNA & Cell Biology; Molecular Carcinogenesis; Genes, Chromosomes & Cancer; Molecular & Cellular Biology; Chemistry & Biology; Cell Growth & Differentiation; Critical Review of Eukaryotic Gene Expression; Mol. Cell. Biol. Res. Comm.; IUBMB-Life; Current Molecular Medicine; Molecular Pharmacology; Current Molecular Medicine, Molecular Cell; Cell Death and Disease; Journal of Experimental Medicine; Current Signal Transduction Therapy; Immunity; Proceedings of the National Academy of Sciences; Gastroenterology; Cell Metabolism; Oncogene; Free Radical Biology and Medicine; Cancer Discovery

U.S. Patents:	**Examples include**
6,863,888 (US)	Oncoprotein protein kinase
5,643,720 (US)	Method of inhibiting transcription utilizing nuclear receptors
5,534,426 (US)	Oncoprotein protein kinase
4,601,978 (US)	Mammalian metallothionein promoter system
7,189,832 (US)	Gamma Subunit of Cytokine Responsive IκB Alpha Kinase Complex and methods
7,314,615 (US)	IκB Kinase-β (IKKβ) Binding Antibodies and Methods of Using Same
7,319,134 (US)	Regulation of Transcription Factor, NF-IL6/ LAP
7,388,071 (US)	Methods for Identifying and Using IKK Inhibi tors
7,399,606 (US)	Methods for Identifying IκB Kinase (IKK) Inhibitors
7,491,506 B2	(US) Inhibition of B-cell Maturation and Anti body Production
7,695,921 (US)	Method for Detecting the Presence of Pros tate Cancer

Honors (Limited listing only)

1984 – 1987	Searle Scholars Award
1990	Oppenheimer Award for Excellence in Research from Endocrine Society
1999 – 2008	Frank and Else Schilling-American Cancer Society Research Professor
2005	Elected Member, National Academy of Sciences
2010	The Harvey Prize in Human Health
2011	Elected Member, Institute of Medicine, The National Academies

Publications (Limited listing only)

Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature. 2012 Nov 8;491(7423):254-8.

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Monica Guma M, Stepniak D, Shaked H, Spehlmann ME, Shenouda S, Cheroutre H, Vicente-Suarez I, Eckmann L, Kagnoff MF, Karin M. Constitutive intestinal NF-κB does not trigger destructive inflammation unless accompanied by MAPK activation. J Exp Med 2011 208:1889-1900

Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature. 2001 Mar 1;410(6824):37-40.

Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol. 2000;18:621-63.

Liu ZG, Hsu H, Goeddel DV, Karin M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. Cell. 1996 Nov 1;87(3):565-76.

Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta. 1991 Dec 10;1072(2-3):129-57.

Angel P, Imagawa M, Chiu R, Stein B, Imbra RJ, Rahmsdorf HJ, Jonat C, Herrlich P, Karin M. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell. 1987 Jun 19;49(6):729-39.

Inflammation and Cancer: Effects, Mechanisms and Therapeutic Implications

Michael Karin

A century and a half ago, equipped with what was then state-of-the-art technology – a microscope and histochemical stains, Rudolph Virchow made the astounding finding that most cancers contain inflammatory infiltrates, an observation that led him to postulate that cancer may be caused by constant irritation. Indeed, the word tumor, meaning "swelling", that is used describe cancer, also refers to one of the five cardinal signs of inflammation. Although it has been known to many other pathologists, that followed Virchow, that solid malignancies contain inflammatory infiltrates composed of monocytes, macrophages, neutrophils and different types of lymphocytes, the suggestion that inflammation and cancer are mechanistically linked has been largely ignored until twenty years ago when newly emerging epidemiological data led to the estimate that about 15% of all cancer-related deaths are linked to persistent infections, such as Helicobacter pylori and Hepatitis B and C viruses, and chronic inflammatory diseases, such as ulcerative colitis. Much of the delay in recognizing and studying the role of inflammation in tumorigenesis has probably been due to the view that cancer is a cell autonomous process governed by activation of oncogenes and loss of tumor suppressors. This dogma, of course, has been dropped in recent years and it is now well acknowledged that the tumor microenvironment also plays a very important role in cancer development and progression and inflammation has been recognized as an enabling characteristic of cancer. Major components of the tumor microenvironment are inflammatory cells, such as tumor associated macrophages (TAM), which play multiple pro-tumorigenic functions, as demonstrated by the Pollard and Montovani groups, including the production of inflammatory cytokines. The general view regarding the role of such cytokines in tumor development and progression has also seen a major revision. Initially, it was expected that the prototypical inflammatory cytokine TNF (tumor necrosis factor) plays a major role in cancer control, as its namesake indicates. Although in high doses TNF can be used in cancer therapy (albeit to a rather limited extent due to its toxicity), chronic production of low amounts of TNF promotes tumor development, as first shown by Balkwill and co-workers

in skin cancer. Other members of the TNF family, lymphotoxin (LT) and RANK ligand (RANKL) produced by B and T lymphocytes, also play important pro-tumorigenic functions in prostate and breast cancers, respectively, as shown in my laboratory. Another very important tumor promoting cytokine affecting the development of both colon and liver cancers is IL-6, whose pro-tumorigenic function was first detected in mouse models but is now supported by strong circumstantial evidence obtained in human studies.

Despite the accumulation of multiple lines of evidence supporting the pathogenic function of inflammatory processes in tumorigenesis, it has not been too obvious what comes first – inflammation or cancer? It is now clear that the answer to this "chicken and the egg" question is – both. We first addressed this question in the classic model of colitis associated cancer (CAC), a form of colorectal cancer (CRC), that appears in patients suffering from ulcerative colitis or Crohn's disease and accounts for about 2% of all cases of CRC. By genetic ablation of IKKβ, a protein kinase that is required for activation of transcription factor NF-κB, a master regulator of inflammatory processes and cell survival, in either intestinal epithelial cells (IEC) or myeloid cells, we have shown that activation of NF-κB in both cell types, which occurs prior to the appearance of malignant colonic tumors, is critical for CAC development. Whereas in pre- malignant IEC, in which β catenin signaling has been activated by oncogenic mutations, IKKβ-driven NF-κB exerts its tumor promoting function through the upregulation of genes that maintain cell survival, in lamina propria myeloid cells NF-κB activation controls the production of tumor promoting cytokines, including TNF and IL-6, that drive the proliferation of pre-malignant IEC. In addition, persistent inflammation can also accelerate the initiation of CAC through upregulation of inducible nitric oxide synthase (iNOS), an enzyme whose product can cause nitrosative stress and genomic instability. However, inflammation can also be the consequence of cancer. Indeed, in the 98% of CRC that does not develop in the context of pre-existing inflammation, we found that formation of colonic adenomas triggers an inflammatory response, referred to as tumor-elicited inflammation, that drives adenoma to carcinoma progression. Tumor-elicited inflammation in CRC occurs early in the tumorigenic pathway and is due to a localized loss of the intestinal permeability barrier, resulting in invasion of the adenomas by commensal microbes or their components (e.g. endotoxin) that trigger the activation of Toll-like receptors and induce the production of the inflammatory cytokine IL-23 by TAMs and intra-tumoral dendritic cells. IL-23 fulfills its pro-tumorigenic function through the upregulation of two other inflammatory cytokines: IL-17 and IL-6. Importantly, elevated expression of a so-called "IL-23-Th17" signature in stage I and II human CRC, as found by Galon and Fridman, is a bad prognostic indicator that is associated with a marked decrease in disease free survival. Two other cancers where inflammation can act both before and after tumor development are liver and pancreatic cancers, two of the most fatal cancers that are refractory to most currently available therapeutics. Pre-existing inflammation, hepatitis caused by viral infections (HBV, HCV), alcohol exposure or excessive consumption of fatty foods or chronic pancreatitis with unknown etiology can greatly increase liver and pancreatic cancer risk. As we first found in mouse models of liver cancer, a major tumor promoting mechanism that can act in both organs is "death-induced inflammation" which is caused by chronic tissue damage. Another important pro-tumorigenic mediator acting in the liver, is LT whose expression can be induced upon HCV infection through a mechanism that involves the IKKβ-dependent activation of NF-κB. The role of NF-κB in liver cancer development, however, is somewhat complex as strong inhibition of NF-κB can augment cell death and thereby lead to "death-induced inflammation" and enhanced tumorigenesis.

While the nascent "inflammation and cancer" field has grown rapidly in the past 10 years, it has yet to reach maturity. It is also facing major challenges, including the therapeutic application of the basic knowledge discussed above. It is currently well established that broad acting anti-inflammatory drugs such as aspirin can reduce cancer risk. The question is whether more specific and more potent anti-inflammatory drugs will be more effective than aspirin? However, the cost associated with such drugs prohibits their use in cancer prevention and economic considerations restrict their application to cancer treatment. In addition to increasing the use of broad acting and cheap anti-inflammatory drugs and anti-oxidants in cancer prevention, I firmly believe that newly approved anti-cytokine drugs, such as neutralizing antibodies and decoy receptors that target IL-6, IL-17 or IL-23 as well as small molecule inhibitors of

Th17 differentiation need to be evaluated for their efficacy in conjunction with currently approved anti-cancer drugs. By inhibiting pro-tumorigenic inflammation, such drugs may greatly enhance the cytocidal effect of conventional therapeutics. In addition, drugs that target pro-tumorigenic cytokines may increase the efficacy of newly developed therapies, such as the anti-CTLA-4 and anti-PD1 antibodies that potentiate the activation of tumor-killing cytotoxic T cells. It is my hope that in the next five years we will see much progress in the translational arena, but this will require a concerted effort between academia, drug companies and regulatory agencies.

References:

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Naugler, W.E., Sakurai, T., Kim, S., Maeda, S., Kim, K., Elsharkawy, A.M., Karin, M. (2007). Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 317:121-124.

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Tan, W., Zhang, W.Z., Strasner, A., Grivennikov, S.I., Cheng, J.Q., Hoffman, R.M., Karin, M. (2011) Tumour-infiltrating T regulatory cells stimulate mammary cancer metastasis through RANKL-RANK signalling. Nature 470: 548-553.

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Grivennikov, S., Wang, K., Mucida, D., Stewart, C-A., Schnabl, B., Jauch, D., Taniguchi, K., Yu, G., Österreicher, C.H., Hung, K.E., Datz, C., Feng, Y., Fearon, E.R., Oukka, M., Tessarollo, L., Coppola, V., Yarovinsky, F., Cheroutre, H., Eckmann, L., Trinchieri, G., Karin, M. (2012) Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature in press

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»

Programm des Wissenschaftlichen Symposiums 2013

Program of the Scientific Symposium 2013

Wednesday, January 30, 2013

12:00 - 13:30 *Registration / Sandwich Lunch*

13:30 - 15:00 **Welcome: Markus G. Manz / Klaus Rajewsky**

Cancer genetics and gene function
Chair: Josef Jiricny, Zurich

Margaret A. Goodell, Houston
De novo DNA methyltransferases in normal and malignant hematopoiesis

Riccardo Dalla-Favera, New York
Pathogenetic and therapeutic insights from the genome of diffuse large B cell lymphoma

Hai Yan, Durham
Glioma genomics and its implications in neuro-oncology

15:00 - 15:30 *Coffee break*

15:30 - 17:30 **Stem cell biology**
Chair: Lukas Sommer, Zurich

Jeremy N. Rich, Cleveland
Partners in crime: Cancer stem cells and the tumour microenvironment

Hans Clevers, Utrecht
Lgr5 stem cells in self-renewal and cancer

Fiona M. Watt, Cambridge
Cancer as a desease of cellular miscommunication

John E. Dick, Toronto
Stem cells in cancer: Are they relevant

17:30 - 18:30 *Apéro*

19:00 - 20:00 **Charles Rodolphe Brupbacher Public Lecture**
Aula of the University of Zurich, Main Building, Rämistrasse 71, 8006 Zurich
Chair: Paul Kleihues, Zurich

Gottfried Schatz, Basel
Die tragische Substanz - Wie genetische Fehler Alterung und Krebs bewirken

Thursday, January 31, 2013

08:00 - 08:30 *Registration*

08:30 - 09:30 **RNA biology and cancer**
Chair: Susan M. Gasser, Basel

Carlo M. Croce, Columbus
Causes and consequences of microRNA dysregulation in cancer

Pier Paolo Pandolfi, Boston
The non-coding RNA revolution in medical research

09:30 - 10:30 **Cancer and inflammation (1)**
Chair: Klaus Rajewsky, Berlin

Yinon Ben-Neriah, Jerusalem
Inflammatory control of colorectal cancer progression

Zena Werb, San Francisco
Role of innate and acquired immune system in mammary development and breast cancer

10:30 - 11:00 *Coffee break + Posters*

11:00 - 12:15 **Cancer and inflammation (2)**
Chair: Klaus Rajewsky, Berlin

Ruslan Medzhitov, New Haven
A mechanism of communication between tumour cells and macrophages

Michael Karin, San Diego
Tumour elicited inflammation and malignant progression in colorectal cancer

12:15 - 14:00 *Lunch + Coffee + Posters*

14:00 - 15:00 **Tumour cell metabolism**
Chair: Michael Hengartner, Zurich

Karen H. Vousden, Glasgow
The role of p53 in metabolic adaptation and survival

Giudo Kroemer, Villejuif/Paris
Premortem autophagy and ER stress as immunogenic signals in cancer therapy

15:00 - 16:30 **Novel approaches to cancer diagnostics and therapy**
Chair: Markus G. Manz, Zurich

Maria Blasco, Madrid
Role of telomeres in cancer and aging

Andreas Trumpp, Heidelberg
Circulating metastasis-initiating cells in breast cancer

Ravi Majeti, Stanford
Targeting CD47 with blocking monoclonal antibodies in human hematologic malignancies

16:30 - 17:00 *Break*

17:00 - 18:30 **Award ceremony**
Charles Rodolphe Brupbacher Prize for Cancer Research 2013

Friday, February 1, 2013

08:30 - 09:30 **Breakthroughs in cancer therapy (1)**
Chair: Robert D. Schreiber, St. Louis

Ralf C. Bargou, Würzburg
T cell engaging antibodies for cancer therapy

Carl H. June, Philadelphia
Engeneered T cell therapy for cancer

09:30 - 10:00 *Coffee break*

10:00 - 11:00 **Breakthroughs in cancer therapy (2)**
Chair: Lars E. French, Zurich

Menno A. de Rie, Amsterdam
New developments in the non-surgical treatment of basal cell carcinoma

Reinhard Dummer, Zurich
Melanoma therapy 2013 –
a multidimensional success story

11:00 - 12:00 **Young Investigator Awards**
Referees: Ralf C. Bargou, Carlo M. Croce, Bernhard W. Stewart, Fiona M. Watt
Awards presented by Mme. F. Brupbacher

Abstracts
Eingeladene Redner

Abstracts
Invited Speakers

Die tragische Substanz. Wie genetische Fehler Alterung und Krebs bewirken

Gottfried Schatz, Basel

Lebende Zellen sind die komplexeste Substanz, die wir bisher im Universum gefunden haben. Unser Körper beherbergt sie in Form von etwa 10'000 Billionen Zellen, die auf noch nicht genau bekannte Weise miteinander vernetzt sind. Dieses wundersame Zellnetzwerk ist langfristig nicht beständig, weil Sauerstoff seine chemischen Bausteine zerstört und unser genetisches System nicht präzise genug arbeitet, um das geordnete Wachstum der Körperzellen sicherzustellen. Die Folgen sind Altern und Krebs.

Weshalb wählte das Leben unbeständige Bausteine? Als es sich auf unserem Planeten entwickelte, enthielt die Atmosphäre noch kein Sauerstoffgas. Die frühesten Zellen konnten also gefahrlos sauerstoffempfindliche Bausteine wie Fette, Eiweisse oder die Erbsubstanz DNS verwenden. Doch dann begannen einige Lebewesen sich vom Sonnenlicht zu ernähren und setzten dabei als Abfallprodukt Sauerstoffgas frei. Dieses zerstörerische Gas reicherte sich in der Atmosphäre an und löste damit wahrscheinlich ein Massensterben aus, da die Zellen ihre sauerstoffempfindlichen Bausteine nicht plötzlich gegen andere austauschen konnten. Fast alle heutigen Lebewesen besitzen deshalb Schutzmechanismen gegen Sauerstoffgas, die aber keineswegs perfekt sind. Wenn wir sie in einer Maus durch genetische Eingriffe schwächen, erkrankt das Tier oder stirbt bereits im Mutterleib. Aber selbst in gesunden Tieren und Menschen trüben Luftsauerstoff und seine hochreaktiven Stoffwechselprodukte im Verlauf des Lebens Augenlinsen und schädigen Netzhaut, Gehirn und die Erbsubstanz DNS.

Instabilität unseres Körpers rührt auch von der Unvollkommenheit unseres genetischen Systems. Unsere Gene bestehen aus fadenförmigen DNS-Molekülen, in denen vier verschiedene Atomgruppen in immer wechselnder Reihenfolge wie in einer Perlenkette aneinandergereiht sind. Gesamthaft sind es stupende 6.4 Milliarden solcher Atomgruppen, die in gedruckter Form eine etwa 100 Meter lange Bücherreihe ergäben. Ihre Reihenfolge im DNS-Faden ist eine Buchstabenschrift, in der unser genetisches Erbe niedergeschrieben ist. Teilt sich eine Zelle, muss sie diese gigantische Informationsmenge getreulich kopieren und an die Tochterzellen weitergeben. Jeder Kopierfehler führt dabei zu einer Mutation, welche die Tochterzellen schädigen kann. Zellen verwenden deshalb ausgeklügelte Mechanismen, um solche Kopierfehler zu vermeiden oder, sollten diese dennoch auftreten, zu korrigieren. Damit erreichen sie, dass bei jeder Zellteilung nur jeder millionste DNS-Buchstabe falsch kopiert wird. Dies mag beeindruckend klingen, doch im Verlauf unseres Lebens ereignen sich in unserem Körper schätzungsweise 10(16) (zehntausend Billionen) Zellteilungen, sodass jedes Gen etwa 10(10) (zehn Milliarden) Chancen für eine Mutation hat. Zu unserem Glück reguliert die Natur jeden wichtigen Wachstumsschritt über mehrere Gene, sodass in einer Zelle mindestens ein Dutzend Gene mutieren müssen, bevor die Zelle unkontrolliert zu wuchern beginnt. Ein vielzelliges Lebewesen - wie z.B. ein Mensch - wäre ohne diese mehrfachen Kontrollen gar nicht lebensfähig. Dennoch können diese Kontrollen versagen, sodass jährlich etwa sechs Millionen Menschen an Krebs erkranken. Altern und Krebs sind tragische Folgen unserer Komplexität, der wir unser Menschsein verdanken.

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De novo DNA methyltransferases in normal and malignant hematopoiesis

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DNA methylation plays a central role in regulating gene expression during development, and is known to be disturbed in a variety of malignancies. The mechanisms through which aberrant DNA methylation contributes to malignancy development, and through which hypomethylating agents exert their effects, are poorly understood. We have studied *de novo* DNA methylation in murine hematopoietic stem cells (HSCs) as a model to address some of these fundamental questions. Murine HSCs express high levels of both *de novo* DNA methyltransferases Dnmt3a and Dnmt3b. Using conditional knockout mice, we have focused on the role of Dnmt3a in murine hematopoiesis, observing that in the absence of Dnmt3a, HSC self-renewal is dramatically enhanced at the expense of differentiation. Serial stem cell transplantation augments this effect, such that phenotypically normal HSCs that fail to differentiate accumulate to high levels. Paradoxically, DNA methylation, examined genome-wide, was both increased and decreased in Dnmt3a knockout HSCs, with CpG islands preferentially hyper-methylated, similar to the pattern of DNA methylation alterations in malignancies. The differentiated progeny of Dnmt3a knockout HSCs exhibited aberrant continued expression of stem cell-specific genes that are normally repressed during differentiation (1). While mutations in *DNMT3A* are prevalent in human acute myeloid leukemia (2), we observed no frank leukemia developing in the mice within the timeframe initially examined. We have now examined HSC-specific Dnmt3b knockout mice, and Dnmt3a-Dnmt3b double knockout HSCs. In absence of both *de novo* DNA methyltransferases, the HSCs accumulate even more dramatically than in the *Dnmt3a* knockout, even though loss of *Dnmt3b* alone has minimal impact. Introduction of oncogenes into the Dnmt3a knockout HSCs decreases the time to malignant transformation compared to the oncogene or *Dnmt3a* knockout alone. We will discuss these recent observations, along with the implications these findings have for understanding mutations of DNA methyltransferases found in human hematologic malignancies.

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Pathogenetic and therapeutic insights from the genome of diffuse large B cell lymphoma

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Diffuse large B cell lymphoma (DLBCL) represents the most common form of B cell-derived non-Hodgkin lymphoma (B-NHL), accounting for ~30% of the *de novo* diagnoses and also arising as a frequent clinical evolution of follicular lymphoma, the second most common type of B-NHL. The molecular pathogenesis of DLBCL is associated with a number of genetic lesions that appear in part to distinctly segregate with individual phenotypic subtypes of this malignancy, which appear to derive from germinal center B cell-like or from post-germinal center B cells resembling in vitro activated B cells, suggesting that these two disease groups utilize distinct oncogenic pathways. However, the lesions that have been identified so far likely may represent only a fraction of those necessary for malignant transformation. Thus, the identification and functional characterization of the entire set of structural alterations present in the DLBCL genome is required for a complete understanding of its pathogenesis and for the development of rationally targeted therapeutic approaches. Toward this end, we have integrated whole-exome sequencing analysis and copy number variation analysis for a comprehensive definition of the DLBCL coding genome. The results have identified a novel set of recurrent genetic lesions, which, in turn, identify novel altered pathways In DLBCL, including both shared and subtype-specific ones.

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Giloma genomics and its implications in neuro-oncology

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Mutations in the critical chromatin modifier *ATRX* and mutations in *CIC* and *FUBP1*, which are potent regulators of cell growth, have been discovered in specific subtypes of gliomas, the most common type of primary malignant brain tumors. However, the frequency of these mutations in many subtypes of gliomas, and their association with clinical features of the patients, is poorly understood. Here we analyzed these loci in 363 brain tumors. *ATRX* is frequently mutated in grade II-III astrocytomas (71%), oligoastrocytomas (68%), and secondary glioblastomas (57%), and *ATRX* mutations are associated with *IDH1* mutations and with an alternative lengthening of telomeres phenotype. *CIC* and *FUBP1* mutations occurred frequently in oligodendrogliomas (46% and 24%, respectively) but rarely in astrocytomas or oligoastrocytomas (<10%). This analysis allowed us to define two highly recurrent genetic signatures in gliomas: *IDH1/ATRX* (I-A) and *IDH1/CIC/FUBP1* (I-CF). Patients with I-CF gliomas had a significantly longer median overall survival (96 months) than patients with I-A gliomas (51 months) and patients with gliomas that did not harbor either signature (13 months). The genetic signatures distinguished clinically distinct groups of oligoastrocytoma patients, which usually present a diagnostic challenge, and were associated with differences in clinical outcome even among individual tumor types. In addition to providing new clues about the genetic alterations underlying gliomas, the results have immediate clinical implications, providing a tripartite genetic signature that can serve as a useful adjunct to conventional glioma classification that may aid in prognosis, treatment selection, and therapeutic trial design.

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Partners in crime: tumor stem cells and the tumor microenvironment

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Cancer research has rediscovered the complexity of nervous system cancers through the incorporation of cellular heterogeneity into tumor models with cellular subsets displaying stem cell characteristics. The cancer stem cell hypothesis posits that tumor cells are organized in a hierarchy with cancer stem cells at the apex and functionally defined by the ability to self-renew and propagate tumors similar to the parental tumors from which they are derived. Cancer stem cells remain controversial due to unresolved relationships with cell-of-origin, frequency, and defining universal markers but it has been nearly impossible to create absolute rules for even a single cancer type so it is likely that the cancer stem cell phenotype can be acquired through convergent evolution and may be governed by variable rules based on tumor type, grade, course, and therapeutic treatment. Cancer stem cells have been studied in multiple cancer types, most heavily in leukemias, glioblastomas, breast and colon cancers. Cancer stem cells also yield the non-tumorigenic tumor bulk cells that display a more differentiated phenotype. The ability to prospectively isolate and interrogate cancer stem cells is defining molecular mechanisms responsible for the tumor maintenance and growth. The clinical relevance of cancer stem cells has been supported by cancer stem cell resistance to cytotoxic therapies and promotion of tumor angiogenesis. Cancer stem cells reside in specific functional niches in perivascular and hypoxic niches that may offer the ability to disrupt tumor maintenance and therapeutic resistance through targeting the niche. The cancer stem cell phenotype is regulated by both cell intrinsic and micro environmental influences that may further increase the complexity of tumor modeling. Notably, cell lines and standard culture conditions used in preclinical studies fail to recapitulate the tumor heterogeneity and do not predict patient responses, supporting the development of improved tumor models. The study of cellular heterogeneity and cancer stem cells has already yielded novel molecular targets and pathways that are amenable to therapeutic targeting, perhaps permitting attenuation of tumor resistance. The conventional pyramidal unidirectional differentiation cascade with cancer stem cells at the apex has been called into question by studies demonstrating plasticity of the cancer stem cell phenotype, thus suggesting that targeting only cancer stem cells will likely fail to cure patients and require simultaneous targeting of cancer stem cells and the bulk tumor. In conclusion although the field of cancer stem cell biology is relatively young, continued elucidation of the tumor hierarchy holds promise for the development of novel patient therapies.

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Lgr5 stem cells in self-renewal and cancer

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The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined *Lgr5* as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of *Lgr5* in cycling, columnar cells at the crypt base. Using an inducible *Cre* knock-in allele and the Rosa26-*LacZ* reporter strain, lineage tracing experiments were performed in adult mice. The *Lgr5*$^{+ve}$ crypt base columnar cells generated all epithelial lineages throughout life, implying that it represents the stem cell of the small intestine and colon. Similar obserations were made in hair follicles and stomach epithelium.

Single sorted *Lgr5*$^{+ve}$ stem cells can initiate ever-expanding crypt-villus organoids in 3D culture. Tracing experiments indicate that the *Lgr5*$^{+ve}$ stem cell hierarchy is maintained in these organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche. The same technology has now been developed for the *Lgr5*$^{+ve}$ stomach stem cells.

Intestinal cancer is initiated by *Wnt* pathway-activating mutations in genes such as *APC*. As in most cancers, the cell of origin has remained elusive. Deletion of *APC* in stem cells, but not in other crypt cells, results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the 'cancer stem cell'-concept.

Fate mapping of individual crypt stem cells using a multicolor *Cre*-reporter revealed that, as a population, *Lgr5* stem cells persist life-long, yet crypts drift toward clonality within a period of 1-6 months. *Lgr5* cell divisions occur symmetrically. The cellular dynamics are consistent with a model in which the resident stem cells double their numbers each day and stochastically adopt stem or telomerase activator fates after cell division. *Lgr5* stem cells are interspersed between terminally differentiated Paneth cells that are known to produce bactericidal products. We find that Paneth cells are CD24+ and express *EGF, TGF-a, Wnt3* and the Notch ligand Dll4, all essential signals for stem-cell maintenance in culture. Co-culturing of sorted stem cells with Paneth cells dramatically improves organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous *Wnt*. Genetic removal of Paneth cells *in vivo* results in the concomitant loss of *Lgr5* stem cells. In colon crypts, CD24+ cells residing between Lgr5 stem cells may represent the Paneth cell equivalents. We conclude that *Lgr5* stem cells compete for essential niche signals provided by a specialized daughter cell, the Paneth cell.

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Cancer as a disease of cellular miscommunication

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Multi-layered epithelia such as the epidermis and oral mucosa are maintained throughout adult life by self-renewal of stem cells and differentiation of their progeny. It is widely assumed that the stem cell compartment drives tumour formation, since the differentiating cells do not divide. Nevertheless, my lab has obtained evidence from a number of different experimental mouse models that both stem cells and differentiating cells can contribute to tumour development. The underlying mechanisms involve short and long range communication between differentiating and dividing epithelial cells, stromal fibroblasts and different populations of bone marrow derived cells such as macrophages. Our observations highlight the importance of cell communication in normal tissue homeostasis and suggest new ways to intervene to prevent cancer progression.

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Stem cells in cancer: Are they relevant

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The cellular and molecular basis for intra-tumoral heterogeneity is poorly understood. Tumor cells can be genetically diverse due to mutations and clonal evolution resulting in intra-tumoral functional heterogeneity. Often proposed as mutually exclusive, cancer stem cell (CSC) models postulate that tumors are cellular hierarchies sustained by CSC heterogeneity due to epigenetic differences (i.e. long term tumor propagation only derives from CSC). There is strong evidence for the CSC model in acute myelogenous leukemia (AML). We have recently developed gene signatures specific to either AML leukemia stell cells (LSC) or normal hematopoetic stem cells and found they share a set of genes that define a common stemness program. Only these stem cell related gene signatures were significant independent predictors of patient survival in large clinical databases. Thus, determinants of stemness influence clinical outcome of AML establishing that LSC are clinically relevant and not artifacts of xenotransplantation. In an effort to determine if the clonal evolution and CSC models of cancer can be unified we have carried out two different studies examining CSC from both the functional and the genetic level. In Ph+ B-ALL, we found that diagnostic patient samples possessed extensive subclonal genetic diversity and through the use of xenotransplant assays, we found that this diversity originated from within the leukemia initiating cells (L-IC). Reconstruction of their genetic ancestry showed that multiple L-IC subclones were related through a complex branching evolutionary process indicating that genetic and functional heterogeneity are closely connected. Our study points to the need to develop effective therapies to eradicate all genetic subclones to prevent further evolution and recurrence. Our second functional/genetic study involved colorectal cancer. Our lab developed a highly reliable xenograft assay for primary colorectal cancer. By combining DNA copy number alteration profiling, targeted and exome sequencing and lentiviral lineage tracing, we followed the repopulation dynamics of many single lentivirus-marked lineages from colorectal cancers through serial xenograft passages. The xenografts were genetically stable on serial transplantation. Despite this genetic stability, the proliferation, persistence, and chemotherapy tolerance of lentivirally marked lineages was variable within each clone. Thus, apart from genetic diversity, tumour cells display inherent functional tumour propagation variability, a mechanism that maximizes tumour fitness contributing both to survival and therapy tolerance.

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Causes and consequences of microRNA dysregulation in cancer

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Since the discovery of *miR-15a* and *miR-16-1* deletions in CLL15, many laboratories around the world have shown miRNA dysregulation in all tumors studied, including the most common, such as lung, breast, prostate and gastrointestinal cancers. Such dysregulation, like the dysregulation of oncogenes and tumor suppressor genes, can be caused by multiple mechanisms, such as deletion, amplification, mutation, transcriptional dysregulation and epigenetic changes.

As miRNAs have multiple targets, their function in tumorigenesis could be due to their regulation of a few specific targets, possibly of even one, or of many targets. A future challenge will be to identify all of the targets of the miRNAs involved in cancer and establish their contribution to malignant transformation. An additional challenge will be the identification of all of the miRNAs that are dysregulated by pathways that are consistently dysregulated in various types of human cancers. This point is of particular importance, as instead of focusing on specific alterations in protein-coding oncogenes or tumour suppressor genes – which may be difficult to treat – we could focus on their downstream miRNA targets. If these miRNA targets are crucial for the expression of the malignant phenotype and the cancer cells depend on their dysregulation for proliferation and survival, we can expect that the use of miRNAs or anti-miRNAs will result in tumor regression. Genomic analyses for alteration in miRNA genes or for copy number alterations in various human tumors by deep sequencing is in progress but has not been completed. These studies could provide additional information concerning the involvements of miRNAs in cancer and in many other diseases.

Over the past few years, we have observed a shift from conventional chemotherapy to targeted therapies, and miRNAs and anti-miRNAs will contribute extensively to the latter.

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The non coding RNA revolution in medical research

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We have recently proposed the existence of a previously unrecognized RNA language by which all RNA transcripts communicate in extensive interconnected networks. The lone code currently available to decipher gene function, the triplet amino acid code described by Nirenberg and Leder (1), can only be applied to 2% of the human genome. Recent work by several groups, including ours, has challenged the traditional view of RNAs as solely templates for protein synthesis, and suggested instead a model in which these complex molecules have important protein-independent functions, including a crucial role in post-transcriptional gene regulation (2). Our pioneering research is the first attempt to systematically functionalize the entire transcriptome, which encompasses both protein-coding and non-coding transcripts. Notably, the transcriptome represents the majority of the human genome and is heavily deregulated in cancer.

Our proposed RNA language is based on the hypothesis that transcripts regulate and communicate with each other by competing for common microRNAs. Transcripts acting in this fashion are termed 'competing endogenous RNAs,' or ceRNAs (2). ceRNA-mediated regulation may involve cross-talk between multiple unrelated RNA molecules in complex networks. This ceRNA language imparts a novel trans-regulatory function to transcripts independent of their protein-coding function, and may shed light on regulatory networks that have been overlooked by conventional protein-coding studies.

We originally tested this hypothesis and proved its validity by studying the interaction between the mRNA encoding for the *PTEN* tumor suppressor gene and its closely related pseudogene, *PTENP1*, which we demonstrated acted as a tumor suppressor through this new mechanism (3). We have extended our analysis to mRNAs and identified several new regulators of *PTEN*, such *ZEB2* and *VAPA*, which are novel tumor suppressive molecules whose function was not previously associated with the proto-oncogenic *PI3/AKT* pathway (4,5). More recently, we focused our work on the *BRAF* pseudogene *BRAFps*, and its role in promoting *MAPK* activation through regulation of its parental gene, *BRAF*. *BRAFps* is over-expressed in human cancers, and is oncogenic in mice engineered to express high levels of it.

This transformative new dimension of gene regulation has significant implications for the systematic functionalization of the entire transcriptome, and represents a paradigm shift for biomedical research at large.

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Inflammatory control of colorectal cancer progression

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Inflammation has many faces. It is most commonly observed as an acute reaction in response to pathogen or another insult, or chronically, accompanying infection and remittent inflammatory disease, such as inflammatory bowel disease (1). Yet, there is another type of smoldering inflammation, which is harder to notice or monitor, which appears to underlie some of the major human diseases: cancer, diabetes type 2 and certain neurodegenerative diseases (2) We have developed mouse models of cancer based on inducible CKIα knockout (3), which exhibit smoldering inflammation, and demonstrate how a low grade, infiltrate-free inflammatory reaction to persistent DNA damage response translates to an aberrant growth. To that end, we determined the inflammatory repertoire of the knockout mice, demonstrated its association with cellular senescence and showed how in the absence of p53, a senescence-inflammatory response (SIR) is converted from a growth inhibitory to growth promoting mechanism, both in vitro and *in vivo*. Anti-inflammatory reagents capable of blocking SIR reverse a tumor-related crypt proliferative phenotype of mutant intestinal organoids in vitro and prevent carcinogenesis in mutant mice. Our studies may explain the anti-carcinogenic effects of non-steroidal anti-inflammatory drugs in human cancer patients (4). Mechanisms and evolutionary aspects connecting inflammation and growth will be discussed.

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Role of innate and acquired immune system in mammary development and breast cancer

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The tumor-promoting role of the immune system has long been recognized. Postnatal development of the mammary gland occurs in an immune competent environment. In cancer the immune environment is compromised allowing neoplastic progression and metastasis to proceed. We have shown that activation of CD4+ T cells by antigen presenting cells (APCs) within the developing mammary and production of IFNγ produced by Th1 effector T cells negatively regulates postnatal mammary gland development. The importance of tissue antigen-presenting cells was demonstrated first, by depletion of $CD11c^{high}/MHCII^{high}$ mammary APCs *in vivo* in mice and *ex vivo* in three dimensional (3D) primary organotypic cultures, which increased ductal invasion and epithelial branching. Second, depletion of alpha/beta T cells, blocking antibodies to MHCII and CD4, and T cell proliferation assays all suggested that regulation of mammary postnatal organogenesis by APCs and CD4+ T cells is antigen-mediated. The production of interferon γ by Th1 effector cells inhibited mammary postnatal organogenesis by acting directly on the mammary luminal epithelial cells. These results suggest a novel regulatory role for the adaptive immune system in normal tissue remodeling, even in the absence of injury or inflammation.
As tumors develop, the inflammatory compartment changes dramatically. Tumor-associated macrophages and APCs and $CD11b^{+}Gr1^{+}$ cells increase tremendously., starting very early before malignant conversion. While the tumor APCs still interact with T-cells, the T-cells no longer are activated for anti-tumor functions. These myeloid cells also increase in tissues distant from the primary tumor setting up sites for metastasis. The expansion of inflammatory cells contributes to the events stimulating tumor growth and metastasis. Characterization of the molecular pathways that regulate these populations will identify pathways and molecules that could be targeted to prevent tumor progression and metastasis.

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A mechanism of communication between tumor cells and macrophages

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Tumor-associated stromal cells, including macrophages, fibroblasts and endothelium play a critical role in tumor development and metastasis. The role of these cell types in tumorigenesis is poorly defined although it is increasingly recognized that stromal cells may represent an attractive target for therapy. Macrophage is one of the most versatile cell types that performs a broad array of functions in most tissues of the mammalian organism. Aside from their role in inflammation and host defense form infections, macrophages have many essential roles in normal physiology and maintenance of homeostasis. The role of macrophages in whole body metabolism has been uncovered in the context of energy imbalance. For example, macrophages can promote pathological sequelae of obesity, such as insulin resistance, by elaborating local inflammatory states. In addition to their well-recognized roles in immunity and inflammation, macrophages function as accessory cells in most tissues. These accessory functions are not yet well defined but are likely to be essential for normal function of most tissues. The same accessory functions can also contribute to pathological processes, such as fibrosis and tumor growth. The focus of this presentation will be on the accessory functions of macrophages, particularly in the context of metabolic control and tumor development.

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Tumor elicited inflammation and malignant progression in colorectal cancer

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Colorectal cancer (CRC) is the third leading cause of cancer related deaths in the US and other Western countries. Whereas 2% of CRC arises in the context of pre-existing inflammatory bowel disease (IBD), especially ulcerative colitis, and is known as colitis associated cancer (CAC), most CRC, including familial and sporadic cases, is found in individuals that do not show any signs of IBD. Yet, expression profiling has revealed the same inflammatory gene signature, which depends on activation of *NF-κB* and *STAT3*, in both CAC and CRC, findings that generate a question regarding the origin of the CRC-elicited inflammatory response. In early experiments we have shown that activation of *NF-κB*, which leads to production of the pro-inflammatory cytokine IL-6, a potent activator of *STAT3*, plays a critical role in the development of CAC. More recently we investigated the origin and role of inflammation in the development and progression of sporadic CRC, most of which is driven by loss of the tumor suppressor APC and activation of the β catenin signaling pathway. We focused our studies on IL-23, a heterodimeric cytokine, composed of a unique p19 subunit and a p40 subunit which it shares with IL-12. As observed by others, we also found that IL-23 expression is strongly elevated in CRC relative to adjacent non-tumor tissue and have extended these findings to the CPC-APC mouse model of colorectal tumorigenesis. In CPC-APC mice, the major source of IL-23 expression in colorectal adenomas are tumor associated macrophages (TAM). Importantly, ablation of IL-23 p19 either in all cells or only in bone marrow-derived cells attenuates the development and slows down the progression of CRC in CPC-APC mice and similar results were observed upon ablation of IL-23 receptor (IL-23R). As IL-23R is not expressed on adenoma epithelial cells, IL-23 must exert its pro-tumorigenic effect via an indirect mechanism. Indeed, IL-23 signaling promotes the polarization of IL-17 producing T cells (Th17) and the production of IL-6, both of which contribute to the development and progression of sporadic colorectal tumors in mice. Importantly, molecular epidemiological studies carried out by Galon, Fridman and their co-workers have revealed that an 'IL-23–Th17' signature that is up regulated in about 10% of human CRC patients, and that the presence of this signature in stage I/II CRC is associated with very poor prognosis and a marked decrease in disease free survival. We investigated the mechanism responsible for the specific induction of IL-23 in TAM and found it to depend on Toll like receptor – MyD88

signaling, which appears to be activated in response to components of the colonic microflora that permeate the adenomas. We also detected eubacterial 16S RNA in both mouse and human colonic adenomas, as well as increased penetrance of bacterial endotoxin into adenomas relative to surrounding non-cancerous tissue. The development of colorectal adenomas in both mice and men is associated with loss of protective mucins and junctional adhesion proteins and this is likely to be the primary mechanism that accounts for the selective entry of microbial products into the tumor and induction of the tumor promoting "IL-23-Th17" signature. Future studies should focus on the therapeutic value of anti-IL-23 or anti-IL-17 interventions and the genetic or environmental causes of the large variation in the magnitude of IL-17 production amongst human CRC patients.

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The role of p53 in metabolic adaptation and survival

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The p53 protein is an important tumor suppressor that functions in a number of ways to prevent cancer development (1). Under conditions of severe or sustained stress, p53 can drive cell death and senescence, thereby removing the damaged and potentially transformed cell. However, recent evidence has also revealed an role for basal p53 levels in helping cells to survive the effects of less intense stress. Under these conditions, p53 plays an important metabolic and anti-oxidant role – so helping cells to cope with the constitutive oxidative stress associated with normal proliferation and growth (2). By helping to promote oxidative phosphorylation and dampen glycolysis, p53 opposes the shifts in metabolism that are characteristic of transformed cells (3). p53 also helps to coordinate the response to starvation by inhibiting cell proliferation and growth, while promoting certain catabolic responses.
The ability of p53 to promote survival and modulate metabolism could contribute to tumor suppression by helping cells to prevent or repair stress and damage. However, it is possible that the inappropriate or deregulated expression of some of these p53 activities may also support cancer progression. We have been investigating the activities of TIGAR, a p53-inducible protein that functions to promote survival. TIGAR can act as a fructose-2,6-bisphosphatase, driving the pentose phosphate pathway, promoting NADPH production to restore reduced glutathione and protecting the cell from ROS-associated apoptosis and autophagy (4,5). Other activities of TIGAR that may also contribute to the survival function are presently under investigation.

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Premortem autophagy and endoplasmic reticulum stress as immunogenic signals in cancer therapy

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The supreme goal of anticancer therapy is the induction of tumor cell death. Physiological cell death, which occurs as a continuous byproduct of cellular turnover, is non-immunogenic or even tolerogenic, thereby avoiding autoimmunity. However, cancer cell death elicited by radiotherapy and some chemotherapeutic agents such as anthracyclines and oxaliplatin can be immunogenic. Immunogenic death involves changes in the composition of the cell surface, as well as the release of soluble immunogenic signals that occur in a defined temporal sequence. This 'key' then operates on a series of receptors expressed by dendritic cells (DC, the 'lock') to allow for the presentation of tumor antigens to T cells and for the initiation of a productive immune response. Immunogenic cell death is characterized by the early cell surface exposure of calreticulin, which determines the uptake of tumor antigens by DC. The late release of the protein high mobility group box 1, which acts on toll-like receptor 4, is required for the presentation of antigens from dying tumor cells. In addition, the release of ATP from dying cells causes the P2RX7 purinergic receptor-dependent activation of the NLRP3 inflammasome in DC, thereby allowing them to release interleukin-1ß and to polarize tumor antigen-specific CD8 T cells towards a Tc1 cytokine pattern. We postulate that the immune system determines the long-term success of anti-cancer therapies, and that this immune response is dictated by immunogenic tumor cell death. Thus, therapeutic failure can result from failure to undergo immunogenic cell death (rather than cell death as such). Agents that fail to induce immunogenic cell death cannot yield a long-term success in cancer therapy. Moreover, tumors that are intrinsically unable to undergo immunogenic cell death are incurable. Importantly, it appears that mitochondrial events determine whether cancer cells die or not in response to chemotherapy, while an endoplasmic reticulum stress response combined with autophagy determines whether this cell death is perceived as immunogenic. We suggest a series of strategies to restore the immunogenicity of cell death in the context of deficient autophagy or endoplasmic reticulum stress.

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Role of telomeres in cancer and aging

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Although cancer and ageing may seem at first glance to be unlikely bedfellows, the origins of their improbable union can be traced back to 1951 when an immortal human cell line was established for the first time; cells that were capable of growing continuously in the laboratory. The same parameters that separate normal cells from their cancer counterparts have proved to be intimately connected to how and why we age.

The complexity of ageing and the biology of cancer do not lend themselves to easy generalizations. Cancer and ageing are both fuelled by the accumulation of cellular damage. Those mechanisms that protect cells from damage simultaneously provide protection against cancer and ageing. By contrast, cancer and longevity require a durable cell proliferation potential

and, therefore, those mechanisms that limit indefinite proliferation (such as telomere shortening and cellular senescence) provide cancer protection, but favor aging
The net balance between these two types of mechanisms, those diminishing the amount of cellular damage (simultaneously protecting against cancer and ageing) and those that prevent excessive cell proliferation (do not normally limit the natural lifespan of animals, but in protected artificial environments may contribute to ageing) must ensure a healthy (cancer-free and ageing-free) lifespan for the majority of individuals during their young and adult life.

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Circulating metastasis-initiating cells in breast cancer

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Metastasis is the foremost cause of cancer-related deaths. Metastatic spread is a complex process initiated by the dissemination, seeding and engraftment of malignant cells in sites distant to the primary tumor (1,2). It has been hypothesized that metastasis-initiating cells (MICs) are present within circulating-tumor-cells (CTCs) in the blood stream of carcinoma patients. Indeed, the presence of CTCs in metastatic patients correlates with decreased overall-survival in several malignancies, including breast cancer. Although these clinical data are consistent with the hypothesis that CTCs contain MICs, their existence, phenotype and activity has never been demonstrated. We present data showing that as low as 1900 CTCs were able to induce metastases in mice. Transplantation of primary patient blood derived CTCs induced metastatic growth in bones and liver, demonstrating the presence of MICs. FACS analysis of primary patient epithelial cell adhesion molecule (EPCAM)+-CTCs revealed heterogeneous inter-patient expression of the metastasis-promoting signaling receptors CD44, CD47 and MET. While the percentage of EPCAM+CD44+CD47+MET+ CTCs in patient blood varied between 1.4 and 44%, metastases both from the original patient and those derived experimentally from CTCs showed high levels of all three receptors. The data provide a first demonstration that EPCAM+-CTCs express CD44, CD47 and MET and contain MICs, providing a molecular basis for the design of diagnostic tools to detect MICs and for developing rational-based approaches to target metastasis in breast cancer.

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Targeting CD47 with blocking monoclonal antibodies in human hematologic malignancies

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Human acute myeloid leukemia (AML) is organized as a cellular hierarchy initiated and maintained by rare self-renewing leukemia stem cells (LSC), which must be eliminated in order to cure the patient. We identified increased expression of CD47 on human AML LSC compared to their normal counterparts. CD47 is a cell surface molecule that serves as the ligand for SIRP-alpha on phagocytes, which in turn transmits a dominant inhibitory signal for phagocytosis. In this way, CD47 essentially functions as a "don't eat me" signal. We hypothesized that disruption of the interaction of CD47 with SIRP-alpha would result in phagocytosis and elimination of AML LSC. We found that blocking monoclonal antibodies directed against CD47 enabled phagocytosis of AML LSC, but not normal CD34+ human bone marrow progenitor cells, by human macrophages *in vitro*. Notably, analogous to a clinical therapy, treatment of human AML-engrafted mice with anti-CD47 antibody eliminated AML cells in the peripheral blood and bone marrow and targeted LSC (1,2). While anti-CD47 antibodies can be effective monotherapy for human AML, such antibodies may be equally, if not more effective as part of a combination strategy. The combination of an anti-CD47 antibody, able to block a strong inhibitory signal for phagocytosis, with a second antibody able to bind an LSC-specific molecule and engage Fc receptors (FcR) on phagocytes, thereby delivering a strong positive signal for phagocytosis, may result in a synergistic stimulus for phagocytosis and specific elimination of AML LSC. We investigated this possibility in human non-Hodgkin lymphoma (NHL), which is targeted by rituximab, an anti-CD20 antibody that recruits immune effector functions by binding to FcR. We identified increased expression of CD47 on human NHL cells, and determined that blocking anti-CD47 antibodies preferentially enabled phagocytosis of NHL cells and synergized with rituximab *in vitro*. Treatment of human NHL-engrafted mice with anti-CD47 antibody reduced lymphoma burden and improved survival, while combination treatment with rituximab led to elimination of lymphoma and cure (3). As hypothesized, these antibodies synergized through a novel mechanism combining Fc receptor (FcR)-dependent and FcR-independent stimulation of phagocytosis that might be applicable to many other cancers. These results establish CD47 as a promising antibody target in human hematologic malignancies and suggest a number of possible strategies for therapeutic targeting of CD47 in the clinic (4). Towards this goal, we have developed a humanized anti-CD47 antibody with potent activity against both AML LSC and NHL cells, and anticipate initiating human clinical trials in the near future.

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T cell engaging antibodies for cancer therapy

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Bispecific antibodies can transiently link tumor cells with otherwise inactive cytotoxic T cells in patients for induction of potent redirected lysis of tumor cells. We have developed so called bispecific single-chain antibodies which are now also called BiTE antibodies (bispecific T cell engager). BiTE antibodies are completely devoid of the constant region of conventional monoclonal antibodies and consist of the variable binding domains of the respective parental antibodies connected by short linker peptides. We could demonstrate in preclinical models that BiTE antibodies have superior anti-tumor efficacy compared to conventional antibody constructs. The clinically most advanced molecule of this novel antibody format is blinatumomab (MT103), a CD19/CD3 bispecific BiTE for the treatment of human B cell malignancies. In first phase I and phase II trials blinatumomab showed strong clinical efficacy in patients with relapsed/refractory non-Hodgkin lymphoma (NHL) and acute lymphoblastic leukemia (ALL).
In a phase I trial in patients with relapsed/refractory B-NHL blinatumomab

showed dose-dependent clinical efficacy with a high rate of partial and complete remissions. There is also initial evidence that blinatumomab has the potential to induce long-term remissions in these patients. In a phase II ALL trial in patients with persistence or relapse of minimal residual disease (MRD) after induction chemotherapy, blinatumomab induced a molecular remission rate of 80%. Again, long-term follow-up showed that blinatumomab has the potential to induce durable remissions in MRD-positive ALL. Based on these results, an international pivotal trial in MRD-positive ALL as well as phase II trials in patients with relapsed/refractory ALL and aggressive lymphoma (diffuse large B cell lymphoma) have been started. Future projects focus on the development of combinatorial approaches and the development of BiTEs in other malignancies.
Conclusion: We have demonstrated the first clinical proof-of-concept of T cell engaging BiTE antibodies for cancer therapy.

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Engeneered T cell therapy for cancer

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We reported promising initial clinical data in 3 patients with chimeric antigen receptor (CAR) T cells targeting CD19 expressed on normal and malignant B cells (CART-19 cells) (1, 2). We now report that 10 patients with relapsed, refractory disease have been treated to date: 9 adults w/CLL and one child w/pre- B cell ALL. Five patients (3 chronic lymphocytic leukemia - CLL, 1 acute lymphocytic leukemia - ALL) had achieved complete response (CR) at the primary endpoint (30 days post infusion) which is sustained and ongoing in all patients (range 2-24 months). 2 CLL patients had a partial response lasting 3 and 5 months, while 3 patients did not respond. In all patients with CR, robust *in vivo* expansion of CART19 cells was observed. Long term peripheral blood persistence of CART-19 cells and CAR19 surface expression was observed in all patients with CR in both CD3+/CD8+ and CD3+/CD4+ subsets. In patients with CR, elimination of peripheral B cells was observed at the time of CART19 *in vivo* expansion. Ongoing B cell aplasia has been documented in each CR patient in both peripheral blood and marrow by flow cytometry. Current data suggests that the magnitude of CART-19 cell proliferation *in vivo* correlates with clinical responses. In contrast, patients with PR showed transient elimination of malignant and normal B cells. Adoptive transfer of CART-19 cells engineered to express CD137 and TcR-zeta signaling domains can result in *in vivo* expansion, homing to disease sites, and long-term functional persistence of CART-19 cells accompanied by ongoing complete clinical responses and long-term B cell aplasia in a substantial fraction of patients with advanced, refractory and high risk CLL and relapsed refractory ALL. A major question facing the field is whether CAR T cells will have clinical activity for non-B cell malignancies.

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New developments in the non-surgical treatment of basal cell carcinoma

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Skin cancer is by far the most common type of cancer in the Western world and skin cancer rates are still rising. In the US alone each year there are over 1 million newly diagnosed cases – more than all other cancers combined. The most frequent type of skin cancer is the basal cell carcinoma (BCC) which accounts for 80%, followed by the squamous cell carcinoma and the melanoma. In the last two years there has been significant improvement of the non-surgical treatment of skin cancer especially.

Recently the relevance of the Hedgehog (Hh) pathway for BCC was demonstrated. The Hh signalling pathway is a key regulator of cell growth and differentiation. The Hh pathway is inactive in most normal tissues, but Hh pathway reactivation has been implicated in the pathogenesis of BCC, basal cell nevus syndrome (Gorlin syndrome), medullobastoma and several other solid cancers. In BCC's most commonly, there is loss of function of patched homologue 1 (*PTCH1*), which normally acts to inhibit the signalling activity of smoothened homologue (*SMO*), a seven-transmembrane protein. Patients with the Gorlin syndrome inherit one defective copy of *PTCH1* and can have hundreds of BCC's. Several *SMO* inhibitors are under development for both topical and systemic use in patients with locally advanced and metastatic BCC. Vismodegib is an oral *SMO* inhibitor that has shown clinical effectivity in advanced BCC patients and Gorlin syndrome patients (1,2). Since therapeutic options were absent for these patients, the introduction of this new class of agents is a major step forward, although the side effects are considerable and frequent. Recently a topical *SMO* inhibitor was introduced that showed clinical efficacy thus circumventing the side-effects of oral *SMO* inhibition (3).

These new developments in the non-surgical treatment of skin cancer have opened new avenues for patients with disfiguring BCC.

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Melanoma therapy 2013 – a multidimensional success story

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In recent years, a number of agents have been shown to improve outcomes for patients with high-risk or advanced metastatic melanoma, including ipilimumab, an immunomodulatory agent, and targeted therapies such as vemurafenib and dabrafenib, which inhibit mutated *BRAF*, and trametinib, an inhibitor of *MEK* signalling (1-5). We now have the opportunity to integrate the knowledge and experience gained from this impressive increase in clinical data into the daily management of patients with metastatic disease. Although the patient's mutational status (*BRAF, c-KIT* and *NRAS*) is a key element for informing treatment with targeted therapies (2-5); clinical information might also prove helpful in guiding appropriate treatment options and sequences. In addition to genotyping, we should also consider the patient's overall prognosis by factoring in clinical parameters such as performance status, whether patients have brain metastases, and laboratory parameters (6). Patients with *BRAF*-mutated melanoma who 'need' a response are candidates for treatment with kinase inhibitors. Patients with smoldering disease are potential candidates for ipilimumab, irrespective of *BRAF* status, and may benefit from salvage therapy with a kinase inhibitor in cases of disease progression.

Despite many unsolved questions, over the last five years we have seen an increase in the average survival time of patients with advanced melanoma at our tertiary referral center in Zurich, underlining the potency of these newly-available medicines. Intelligent clinical trials, including investigations of combination and sequencing strategies, however, are required to further improve the efficacy of available treatments.

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Poster Abstracts

1

A dual role of peroxiredoxin 6 in skin carcinogenesis

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The antioxidant enzyme peroxiredoxin 6 (Prdx6) is a key regulator of the cellular redox balance, particularly under stress conditions. We identified Prdx6 as an important player in different phases of skin carcinogenesis. Loss of Prdx6 in mice enhanced the susceptibility to skin tumorigenesis, whereas overexpression of Prdx6 in keratinocytes of transgenic mice had the opposite effect. The tumor preventive effect of Prdx6, which was observed in a human papilloma virus 8-induced and a chemically-induced tumor model, was not due to alterations in keratinocyte proliferation, apoptosis or in the inflammatory response. Rather, endogenous and overexpressed Prdx6 reduced oxidative stress as reflected by the reduction in oxidized phospholipids in the pro-tumorigenic skin of Prdx6 transgenic mice and the increase in Prdx6 knockout mice. In contrast to its beneficial effect in the control of tumor formation, malignant progression of existing tumors was accelerated by overexpression of Prdx6, revealing a dual function of this enzyme in the pathogenesis of skin cancer. The human relevance of this finding is supported by the strong expression of Prdx6 in keratinocytes of normal human skin and in the tumor cells of squamous cell carcinomas. Our data suggest that activation or inhibition of Prdx6 could be a useful strategy to control different stages of skin carcinogenesis.

2

A switch between DNA polymerases δ and λ promotes error-free bypass of 8-oxo-G lesions

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7,8-dihydro-8-oxoguanine (8-oxo-G) is a highly abundant and mutagenic lesion. Replicative DNA polymerases (pols) are slowed down at 8-oxo-G and insert both correct C and incorrect A opposite 8-oxo-G, but they preferentially extend A:8-oxo-G mispairs. Nevertheless, 8-oxo-G bypass is fairly accurate *in vivo*. Thus, the question how correct bypass of 8-oxo-G lesions is accomplished despite the poor extension of C:8-oxo-G base pairs by replicative pols remains unanswered. Here we show that replicative pol δ pauses in front of 8-oxo-G and displays difficulties extending from correct C:8-oxo-G in contrast to extension from incorrect A:8-oxo-G. This leads to stalling of pol δ at 8-oxo-G after incorporation of correct C. This stalling at C:8-oxo-G can be overcome by a switch from pol δ to pols λ, β or η, all of which are able to assist pol δ in 8-oxo-G bypass by translesion synthesis (TLS). Importantly however, only pol λ selectively catalyzes the correct TLS past 8-oxo-G, while pols β and η show no selectivity and even preferentially enhance incorrect TLS. The selectivity of pol λ to promote the correct bypass depends on its N-terminal domain. Furthermore, pol λ-/- mouse embryonic fibroblast extracts display reduced 8-oxo-G TLS. Finally, the correct bypass of 8-oxo-G in gapped plasmids in mouse embryonic fibroblasts and HeLa cells is promoted in the presence of pol λ. Our findings suggest that even though 8-oxo-G is not a blocking lesion *per se*, correct replication over 8-oxo-G is promoted by a novel pol switch between pols δ and λ.

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3

DNA replication stress in embryonic stem cells?

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Embryonic stem cells (ESCs) have the ability to self-renew and to differentiate into multiple cell types. Conditional inactivation of ATR - the central factor activated by DNA replication stress - rapidly leads to stem cell depletion, suggesting that stem cells experience replication stress during proliferation(1). We show that ESCs exhibit high levels of endogenous γH2AX foci - a marker for replication stress and DNA damage - when compared to differentiated and committed cell lines. Upon induction of differentiation, γH2AX foci disappear as soon as stem cell markers are lost, well before cells stop proliferating. Thus, stemness seems to be inherently associated with genotoxic stress. ESCs lack 53BP1 foci, a specific marker for DNA double strand breaks, but exhibit strong staining for RPA and Rad51 - both single stranded (ss)DNA-binding proteins - which is further indicative of replication stress. Preliminary experiments to visualise replication intermediates in ESCs reveal accumulation of ssDNA gaps. Accordingly, low doses of camptothecin - a topoisomerase-1 inhibitor that induces ssDNA nicks and causes replication fork slowdown in cancer cells - has marginal effects on ESCs, where such lesions are presumably already present. In line with our previous work on CPT-treated cancer cells(2), poly ADP ribose polymerase inhibition impedes fork progression in ESCs and leads to extensive breakage of replicating chromosomes. We are currently investigating the source of these frequent strand discontinuities in ESCs and the molecular mechanisms evolved by these cells to tolerate them during DNA replication.

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4

Aldara treatment of human and murine squamous cell and basal cell carcinomas

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Aldara (5% imiquimod, a TLR-7 agonist, in cream) is a local therapy for basal cell carcinoma (BCC), non-invasive squamous cell carcinoma (SCC) and actinic keratosis with an astonishingly high efficacy. The increased infiltration of the lesion by TLR7-positive plasmacytoid dendritic cells (pDC) supports the idea that signaling through TLR7 is crucial for therapy. However, our results show that Aldara induces a plethora of different TLR7- and imiquimod-independent immune defense pathways, including the activation of the inflammasome, keratinocyte pyroptosis and the production of pro-inflammatory cytokines (IL-6, IL-17, IL-23). The vehicle cream induces most of these events, although imiquimod seems to have an amplifying effect on particular processes. This means that Aldara-induced tumor regression may depend on the concerted action of processes initiated by the vehicle plus such initiated by imiquimod. Moreover, we recently showed that treatment of human BCCs with Aldara results in an upregulation of MHC-I and is accompanied by increased peritumoral infiltration of CD8+ T cells. These findings suggest that, in addition to innate immunity, adaptive immunity may contribute to the efficacy of Aldara treatment of non-melanocytic skin cancer (NMSC). To further investigate which pathways are crucial in the control of NMSC, we are currently using a murine model for SCC (K14-HPV8 E6), in which the HPV8 E6 gene is expressed under the control of the keratin-14 promoter, which activates expression of this gene in basal keratinocytes, leading to the spontaneous development of skin tumors.

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5

Identification of new cellular targets for lung cancer therapy

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From *in vitro* studies it is known that inhibition of REV3, which is the catalytic subunit of the translesion polymerase ζ (zeta), reduces formation of resistance and increases sensitivity of human cells to cisplatin. Recent findings of our laboratory revealed that inhibition of REV3 expression suppresses growth of mesothelioma, lung- and breast cancer cells, whereas growth of control cells is less affected. A "synthetic lethal" interaction exists when deficiencies in two pathways alone can be tolerated but become lethal when combined. Cancer cells are already deficient for cell cycle checkpoint control. Thus, inhibition of DNA repair, e.g. inhibition of Rev3 expression, renders cancer cells extremely sensitive to additional stress by molecular pathways which are yet to be identified. The purpose of this project is to identify genes whose inhibition will not only reduce, but completely abolish cancer cell growth in a REV3-deficient background. We generated clones of a non-small cell lung cancer cell line A549 with reduced REV3 expression and performed sets of global screens to identify targets whose inhibition affects viability of REV3-deficient A549 cells. *In vitro* evaluation of the screening results by siRNA deconvolution confirmed that silencing of some of the hits is synergistic to inhibition of REV3 expression. The used approach will help us to determine the mechanism of the observed preferential growth retardation of the cancer cells and to justify its possible therapeutic application.

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6

Role of 14-3-3 proteins at stalled replication forks

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Problems related with the integrity of DNA replication forks are important for cell viability. In replication checkpoint-deficient budding yeast, disturbances caused by DNA damaging agents possibly result in fork collapse or DNA breaks/rearrangements. Previous studies from our and other laboratories showed that in hydroxyurea-treated yeast cells, 14-3-3 proteins are recruited to DNA replication forks and contribute to control the activity of exonuclease-1, which would otherwise threaten their integrity. These studies also highlighted the fact that additional unknown targets of 14-3-3 proteins contribute to promote fork progression, stability and restart. The overall goal of this work is to determine the molecular mechanism of 14-3-3 proteins function at stalled DNA replication forks.
Our data demonstrate a genetic interaction between BMH1 and DPB3, suggesting a possible role for Dpb3 in 14-3-3-mediated replication fork regulation.

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7

The landscape of cancer genomes reveals correlation between somatic copy number aberrations and fundamental genome structure

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Copy number aberrations (CNA) are a hallmark of cancer genomes. Recurrent CNA hotspots are known to target well defined cancer genes. However, the average extent of CNA encompasses thousands of gene loci, far beyond the involvement of a limited set of strong 'cancer driver' genes. To explore indications for a non-neutral selection for CNA regions, we analyzed a possible relationship between CNA occurrence and local gene density across the genome. We performed a genome-wide analysis of 744,139 somatic CNAs from 19,471 cancer samples, classified into 65 diagnostic types. We observed a significant enrichment for small and extended localized CNA in gene-rich regions, both in terms of gene numbers and coding regions. The 1,488,278 DNA breakpoints that were associated with the CNA also showed a positive correlation with gene-rich regions. However, the frequency of arm-level CNA was negatively correlated with gene numbers on chromosome arms. Notably, when investigating cancer and platform type specific data, we obtained similar results. The landscape of cancer genomes revealed a global correlation between CNA and genome structure and provides support for a non-neutral selection of multiple and/or extended CNA as recurring feature of cancer genome evolution.

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8

Cytotoxic CD8+ T cells regulate myelopoiesis by cytokine signals to bone marrow stromal cells

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Cytotoxic CD8+ T cells (CTLs) play a major role in host defense against intracellular pathogens. However, complete clearance of pathogens and return to homeostasis requires a regulated interplay of innate and acquired immunity. In this study, we show that interferon-gamma (IFNγ) secreted by effector CTLs regulates hematopoiesis and induces myeloid differentiation resulting in an accumulation of myeloid cells. IFNγ did not primarily affect hematopoietic stem cells or progenitors directly, but stimulated mesenchymal stromal cells of the bone marrow (BM) stem cell niche to produce hematopoietic cytokines including interleukin-6. This induced a myeloid bias in hematopoietic stem cells, resulting in proliferation and accumulation of myeloid but not lymphoid progenitors. Our study indicates that CTLs exert an indirect positive feedback on myelopoiesis via BM stromal cells during clearance of an acute viral infection.

9

Cytotoxic T cells induce proliferation of chronic myeloid leukemia stem cells by secreting interferon-gamma

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Chronic myelogenous leukemia (CML) is a clonal myeloproliferative neoplasia arising from the oncogenic BCR/ABL translocation in hematopoietic stem cells (HSCs), resulting in a leukemia stem cell (LSC). Cure of CML depends on the eradication of LSCs. Unfortunately, LSCs are resistant to current treatment strategies. The host's immune system is thought to contribute to disease control and several immunotherapy strategies are under investigation. However, the interaction of the immune system with LSCs is poorly defined. In the present study, we document in a murine CML model that LSCs express major histocompatibility complex and costimulatory molecules and are recognized and killed by leukemia-specific CD8+ effector CTLs *in vitro*. In contrast, therapeutic infusions of effector CTLs into CML mice *in vivo* failed to eradicate LSCs but paradoxically increased LSC numbers. LSC proliferation and differentiation was induced by CTL-secreted interferon-gamma (IFNγ). Effector CTLs were only able to eliminate LSCs in a situation with minimal leukemia load where CTL-secreted IFN-g levels were low. In addition, IFNγ increased proliferation and colony formation of CD34+ stem/progenitor cells from CML patients *in vitro*. Our study reveals a novel mechanism by which the immune system contributes to leukemia progression and may be important to improve T-cell-based immunotherapy against leukemia.

10

Role of the epigenetic modifier EZH2 in melanoma

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Previously, we have described critical molecular pathways that are involved in the development of embryonic neural crest cells. It has become evident that these key molecular players also play roles in neurocristopathies, such as melanoma, a cancer that arises from the melanocytic lineage. Among others, polycomb group proteins are key players that are involved in neural crest development, and presumably also in neurocristopathies. For example, increased expression of the polycomb group protein EZH2 in human cutaneous melanoma is associated with metastasis formation and poor prognosis. EZH2 is a methyltransferase that triple-methylates histone-3 at lysine-27, and thereby induces epigenetic silencing of the corresponding gene. Nevertheless, EZH2-mediated gene regulation has not yet been studied in an *in vivo* melanoma model.
To investigate whether EZH2 plays a functional role in melanoma development, we made use of Tyr::N-RasQ61K Ink4a-/- mice, which develop melanoma within 6 months (1,3). Firstly, we conditionally ablated Ezh2 in such melanoma-developing mice before onset of tumorigenesis by applying the Cre-LoxP system (cKO). Strikingly, cKO mice benefit from Ezh2 ablation, and display longer melanoma-free survival compared to control littermates. On day of sacrifice, cKO mice have significantly less primary tumors than control animals and a complete lack of metastases. Secondly, we conditionally ablated Ezh2 after onset of melanomagenesis. Most remarkably, cKO mice show stabilization and, in some cases, regression of the disease compared to control littermates. These findings demonstrate the crucial requirement of EZH2-mediated gene repression for melanoma development, and make EZH2 an interesting candidate to develop novel melanoma therapies.

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Biochemical analysis of Saccharomyces cerevisiae Mlh1-Mlh3 endonuclease

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The homologous recombination pathway has evolved to repair double strand DNA breaks, which may lead to crossover and non-crossover recombination products. Mitotic cells avoid crossovers in order to maintain the genome stability. In contrast, meiosis favors crossovers, which help generate diversity among the progeny originated from common parents. The main pathway that promotes crossovers during meiotic recombination involves the MutS homologs Msh4-Msh5 (MutS ϒ) and MutL homologs Mlh1-Mlh3 (MutLϒ). These proteins are anticipated to cleave intermediates of homologous recombination in a way that favors crossover outcome. Our primary interest is the role and mechanism of the Mlh1-Mlh3 pathway. It has been proposed that the MutSϒ complex first stabilizes the Holliday junction intermediate and MutLϒ then subsequently resolves it to form crossovers. Surprisingly, recent genetic data from yeast has revealed that Sgs1 and Exo1 show functional interactions with MutLϒ and are also required for producing crossovers. To date, we have successfully produced recombinant MutLϒ and MutSϒ protein complexes and designed various DNA structures, which mimic intermediates formed during homologous recombination. Encouragingly, we saw that both heterodimers bind preferentially Holliday junction intermediates, which is our first step towards understanding this mysterious pathway. Next, we would like to investigate the nuclease activity of MutLϒ on these structures as well as the roles of Sgs1 and Exo1 proteins.

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Metabolic profiling of experimental tumors using hyperpolarized [1-13C] pyruvate: evaluating cell type specificity and effects of tumor environment

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Hyperpolarized 13C magnetic resonance spectroscopy and imaging is a rapidly expanding field with many applications, particularly in the field of cancer. Polarization of a number of enzyme substrates enabled the studies of key steps in glycolysis and tricarboxylic acid cycling [1], with [1-13C]-pyruvate being one of them. In this study we compared the lactate dehydrogenase (LDH) activity by measuring the conversion of [1-13C]-pyruvate to [1-13C]-lactate by (i) using same type of tumor cells growing at different implantation sites (subcutaneous versus orthotopic) and using mice of different genetic background thereby assessing effects of the tumor environment on the enzymatic conversion rate, and (ii) by using different tumor cell lines in order to assess differences in the metabolic status amongst the tumor cell lines. We showed that the LDH forward rate constant (averaged over the tumor volume) for 4T1 tumors implanted either orthotopic in Balb/c or nude Balb/c mice or subcutaneous in nude Balb/c mice does not to depend on the site of implantation and the mouse strain used. By calculating the group statistics irrespectively of tumor size for the different tumor types studied, we found significant differences in the LDH rate constant when comparing subcutaneous C51 and 4T1 tumors as well as orthotopic 4T1 tumors and gliomas. This indicates that the pyruvate processing through the LDH reaction reflects an intrinsic property of the tumor cell line, which at least for 4T1 tumors is not altered during tumor growth.

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Deciphering the transcription (co)factor network of epithelial to mesenchymal transition

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The formation of metastasis from a primary tumor is achieved through a metamorphosis of adherent tumor cells to highly migratory and invasive cells. We are particularly interested in the fundamental questions of how these cells, with an original epithelial imprinting, de-differentiate into metastatic tumor cells with a mesenchymal gene expression profile. During this process, also known as epithelial to mesenchymal transition (EMT), tumorigenic cells undergo a major transcriptional reprogramming that leads to the conversion of benign, adhesive cells to malignant, invasive cells (1). The massive changes in gene expression during EMT argue for a critical role of master regulators that act on the level of chromatin modifications and direct transcriptional control.
To study the transcriptional and epigenetic control of cellular de-differentiation, we induce EMT *in vitro* by the addition of TGFβ to murine epithelial cells and follow the transcriptional and phenotypic changes over time. By combining RNAi screening approach together with microarray analysis and chromatin-immunoprecipitation, we aim to decipher the complex networks driving EMT. By using a phenotypic microscopy-based screen of transcription (co)factors, we identified 270 factors awaiting confirmation. Interestingly, many hits are regulators of DNA or histone modifications, indicating that epigenetics could play a major role during EMT. To study the contribution of these hits to EMT, we will establish a transcription (co)factor network and follow up the hub genes with *in vitro* and *in vivo* experiments. Using this approach, we hope to gain a more global understanding of the processes underlying malignant tumor progression and metastasis.

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Cpt1c depletion protects from tumor growth in the neurofibromatosis type 1 tumor model

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Carnitine palmitoyltransferase 1C (Cpt1c) is a brain-specific metabolic enzyme involved in energy homeostasis (1). We identified Cpt1c as a gene regulated by the p53 transcription factor, suggesting an involvement in cancer. Tumor cells constitutively expressing Cpt1c show increased fatty acid oxidation and increased ATP production (2). We show that expression of Cpt1c is induced by metabolic stresses such as glucose deprivation and hypoxia in a p53 and AMP-activated protein kinase (AMPK)-dependent manner. To better understand the role of Cpt1c in tumorigenesis *in vivo*, we investigated the impact of loss of Cpt1c function in a mouse tumor model. We used the Nf1+/-:p53+/- mice as a tumor model The Nf1:p53 heterozygous mice develop brain tumors and sarcomas with high frequency. These mice were crossed with mice depleted of Cpt1c (Cpt1cgt/gt). The survival of the Nf1+/-:p53+/-:Cpt1c knock out (KO) mice was compared with the Nf1+/-:p53+/-mice, the Cpt1cgt/gt mice and a wild type control (C57BL/6 strain). A higher survival rate and a lower frequency of tumors were observed in the Nf1+/-:p53+/-:Cpt1c KO mice if compared with the Nf1+/-:p53+/- group. Tumors isolated from the Nf1+/-:p53+/- mice showed an increased proliferation rate and high levels of Cpt1c (compared to healthy tissue). Cpt1c overexpression in the tumors correlated with p53 and AMPK activity. Cpt1c expression in the Nf1+/-:p53+/- mice might contribute to a malignant cancer phenotype by helping tumor cells to reprogram their metabolism and by giving resistance to apoptosis and to hypoxic environments.

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Lymphotoxin-beta receptor signaling increases progression of chronic myeloid leukemia in mice

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Despite promising progress in long term treatment of leukemia, the removal of cancer stem cells (CSC), and therefore cure of the disease, remains an unmet need. Numerous studies indicate that CSC are resistant to conventional treatment such as radiotherapy or chemotherapy. Tumor necrosis factors and their receptors (TNFR) regulate cell survival, growth, apoptosis and differentiation. TNFR signaling is of importance in anti-tumor immunity but also contributes to chronic inflammation. Lymphotoxin-beta receptor (LTβR) is one of 25 receptors of the TNFR family. Besides its role in regulating developmental programs and formation of lymphoid organs, LTβR is critically involved in the regulation of the immune response. In this study, we now show that LTβR is expressed on normal hematopoietic stem cells as well as on leukemia stem cells. Myelopoiesis in naïve LTβR -/- mice is comparable to naïve BL/6 mice, including comparable numbers of hematopoietic stem and progenitor cells in the bone marrow. To investigate the role of LTβR signaling in leukemia, we induced chronic myelogenous leukemia (CML) in mice by retroviral transduction of bone marrow progenitor cells with BCR/ABL. Surprisingly, in the absence of LTβR signaling on leukemia stem and progenitor cells, CML developed significantly slower than in control CML mice. Therefore, blocking LTβR signaling may be a strategy to target leukemia stem and progenitor cells directly.

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Dissection of the mechanisms that support tumor-specific immunity upon radiotherapy

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Radiotherapy (RT) is an important treatment for cancer that takes advantage of the higher radio-sensitivity of cancer cells compared to untransformed cells. The main mode of action is thought to be the irreversible damage to tumor cell DNA, but there is accumulating evidence suggesting that RT supports tumor-specific, protective immunity. Using mouse models with transplantable, syngeneic tumors we found that the therapeutic efficacy of a single dose of 10 Gy crucially depended on CD8+ T cells and dendritic cells (DCs). Furthermore, local RT resulted in DC activation, which appeared crucial for the development and/or maintenance of effector function of tumor-specific CD8+ T cells and tumor control. To dissect the mechanisms, we performed microarray analysis. RT impacted on different pathways including the complement system, the IL1/IL6 inflammation cascade and the HMGB1 pathways. All of these three pathways show an early response to the treatment, in particular HMGB1 pathway and complement cascades are up-regulated the same day of RT at the mRNA level. Regarding the complement cascade only the classical and lectin pathways showed *in vitro* up-regulation. We detected increased amounts of activated complement in the entire tumor mass by immunofluorescence early after radiotherapy. These findings suggest that multiple mechanisms contribute to the clinical response to radiotherapy including the involvement of both innate and adaptive response.

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18F-RB390: a novel ligand for imaging the T877A androgen receptor mutation in prostate cancer via positron emission omography (PET)

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Prostate cancer is the most common cancer in men and the second leading cause of cancer deaths. Detecting prostate cancer before its spread is a challenge which impacts upon the patient's survival. Presently, 2-[18F]-fluoro-2-deoxy-D-glucose (18F-FDG) and/or 18F-choline are the most commonly used PET-tracers in oncology yet they do not emphasize the T877A androgen receptor (AR) mutation escaping androgen deprivation treatment. We designed and synthesized fluorinated dihydrotestosterone (DHT) derivatives to target T877A-AR exclusively present in cancerous tissue. We performed *in vitro* binding to select suitable candidates using transfected COS-7 cells with wild-type (wt) or T877A-AR plasmids. Cellular uptake of candidate 18F-RB390 was investigated in selectively transfected COS-7 cells, C4-2 and AR-free PC3 cells. PET-imaging was conducted in C4-2 and PC-3 tumor bearing SCID-mice (n=12). Molecular docking was used to model interactions of RB390 with wt and T877A-AR. RB390 presented a higher relative binding affinity (RBA) (28.1%, IC50=32nM) for T877A-AR than for wt-AR (1.7%, IC50=357nM) compared to DHT (RBA=100%). A small fraction of 18F-RB390 was metabolized using murine liver homogenate (3h), but by 50% using human liver homogenate. 18F-RB390 but not 18F-FDG or 18F-choline accumulated in COS-7 cells transfected with pSG5AR-T877A than with pcDNA3 or pSG5AR-plasmid. Maximal internalized radioactivity was reached within minutes and was reduced with an excess of unlabeled DHT. 18F-RB390 (10MBq) with and without cold DHT was injected iv and PET/CT scanning performed 1h later. Co-administration of cold DHT prevented PET response. Given the differential binding capacity and favorable radioactivity pattern, 18F-RB390 represents a novel imaging ligand with diagnostic potential for prostate cancer.

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Detection and functional portrayal of a novel class of dihydrotestosterone derived selective progesterone receptor modulators (SPRM)

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At present mifepristone (RU 38486), a synthetic steroid, is clinically used as an abortifacient agent to block progesterone receptors (PR). However, this first-generation PR ligand also cross-reacts with other nuclear receptors such as androgen receptor (AR), glucocorticoid receptor (GR) and estrogen receptor. Since the crystal structures of the ligand-binding domain of both human PR and AR share 54% identity, we hypothesized that derivates of dihydrotestosterone (DHT), the endogenous cognate ligand for AR might also bind to PR. Therefore our aim was to identify selective PR ligands with potent antagonistic properties. Various DHT derivates designed and synthesized in our laboratory were investigated using competition-binding assays for AR, PRbeta, GRalpha and mineralocorticoid receptor (MR). Agonistic and antagonistic activities of eight compounds not displacing 3H-DHT from AR were studied by reporter and alkaline phosphatase assays. Six candidates were pan-antagonists, three presented mixed (agonist/antagonist) activities for PRbeta and two compounds, RB142 and RB143, presented a better selectivity with regard to mifepristone by antagonizing PRbeta and MR with

little effect on GRalpha and no effect on AR. Tested on breast cancer cells (MCF-7/T47D), both compounds demonstrated cytostatic properties without apoptosis induction. Using chimeric receptors (Cherry-PRbeta and GFP-AR), we found a selective nuclear translocation of PRbeta by RB142 and RB143 contrasting with DHT, progesterone and mifepristone inducing PRbeta and AR translocations. 3D models of human PR and AR with RB142/143 were achieved to define molecular selectivity keys. In conclusion this study underlines the relevance of screening DHT derivates to discover new selective steroidal PR-antagonists.

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Wild-type ALK and both ALK-R1275Q and ALK-F1174L activating mutations display oncogenic activity in murine neural crest progenitor cells

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Neuroblastoma (NB), a neural crest-derived embryonal tumour, is a heterogeneous childhood malignancy that often presents as an incurable disease. The anaplastic lymphoma receptor tyrosine kinase gene (ALK) was shown to be overexpressed, mutated or amplified in the majority of NB. The most common mutation, ALK-F1174L displayed an efficient transforming activity *in vivo*, and was shown to drive NB formation in transgenic mouse models. We aimed at comparing the oncogenic potential of ALK-wt, with the two most frequent activating mutations ALK-F1174L (only present in familial NB), and ALK-R1275Q (present in familial and sporadic NB). For this purpose, ALK-wt and mutated sequences were stably expressed in the murine neural crest progenitor cells JoMa1.
Only ALK-F1174L conferred tumorigenic potential to JoMa1 cells *in vitro* as measured by clonogenic assays. In contrast, ALK-wt, or ALK-R1275Q expressed in JoMa1 cells were capable of driving tumour formation *in vivo*, after subcutaneous or orthotopic (adrenal gland) implantation. Interestingly, JoMa1-ALK-F1174L displayed an increased *in vivo* tumorigenic potential compared to ALK-wt and ALK-R1275Q, as ALK-F1174L derived tumours grew much faster. Orthotopic tumours and tumour-derived cell lines strongly upregulated c-myc expression, however, they remained dependent on the ALK initiating oncogene, as specific ALK inhibition reduced their viability and completely inhibited their clonogenic capacity *in vitro*. Interestingly, subcutaneous tumours displayed a NB-like phenotype, while orthotopic tumours are under characterisation. This is the first demonstration of an oncogenic activity of ALK-wt and ALK-R1275Q mutation *in vivo*. Thus, in addition to

ALK-F1174L, ALK-wt and ALK-R1275Q mutation may be instrumental in driving NB development.
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Vaccination strategy to induce antigen-specific cytotoxic CD8 T cells (CTL) in bladder mucosa and regression of murine orthotopic bladder tumors

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Most bladder tumors are non-muscular and invasive, with a high propensity to recur and/or progress after local resection. Vaccines against tumor-associated antigens are being developed, although it is as yet unclear how to target vaccine-specific cell mediated immune responses to the bladder. Here, using an adjuvanted human papillomavirus oncogene (E7) vaccine as a model, we compare in mice, different routes of immunization including administration subcutaneously (sc), intra-nasally or intravaginally (ivag). E7-specific CD8 T-cell responses were determined in spleen, lymph nodes and in bladder. Both sc and ivag vaccination induced a similar number of TetE7+CD8+ T-cells in the bladder, while sc immunization induced higher responses in the spleen and intra-nasal immunization was ineffective. Interestingly, this contrasts with vaccine-specific responses measured in the nearby genital mucosa (1), suggesting other integrin/selectin as being responsible for bladder T-cell homing. Indeed, our data shows that only sc and ivag routes induced vaccine-specific CD8 T cells that predominantly express alpha-L-, alpha-4- and E-selectin-ligand that can home into the bladder. Bladder-tumor regression was evaluated by *in vivo* bio-imaging in an orthotopic model with tumor-cells co-expressing E7 and luciferase. A single E7 immunization by either routes conferred full tumor protection in a prophylactic setting. In a therapeutic setting, both ivag and sc vaccination efficiently caused regression of established bladder-tumors in approximately 60% of mice whereas intra-nasal immunization was ineffective. Tumor regression correlated with an increased tumor infiltration of vaccine-specific CD8 T-cell concomitant to decreased regulatory T-cells. Our results shows that either s.c. or ivag vaccination routes may be used to provide anti-tumor responses in the bladder.

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Identification and characterization of "read-through" chimeric transcripts in renal cell carcinoma

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Read-throughs are chimeric transcripts originating from two directly adjacent genes located close to each other on the same DNA strand. Although they are found in transcriptome sequencing data on a regular basis, little is known about their expression patterns and functions in cancer. Using a computational software (FusionSeq 1), specifically designed to call chimeric transcripts, we nominated 324 read-through events in seven human renal cell carcinoma (RCC) tissue samples (five clear cell, one chromophobe and one Xp11 translocation RCC) which were subjected to paired-end whole transcriptome sequencing. Frequently, we identified various isoforms of a given read-through event. In the majority of isoforms, the exon junction was outside the ORFs or produced a frameshift predicting fully or partially conserved parent proteins or isoform degradation. Quantitative PCR analysis revealed two read-throughs that are higher expressed in RCC compared to matched adjacent benign kidney tissue. We show that elevated BC039389-GATM expression is associated with mTOR signaling induction through upregulation of RHEB, the activator of the MTORC1 complex. The second

read-through, KLK4-KRSP1, seems to negatively influence apoptosis signaling by differential regulation of apoptotic (TXN2) and anti-apoptotic factors (TRAIL, TRAIL-R1). An additional read-through, TMED6-COG8, was found expressed at elevated levels in Xp11 translocation RCC compared to clear cell RCC, and may serve as surrogate marker for TFE3 translocation. Our data suggest that the upregulation of read-through RNA chimeras in tumors may have potential cancer-promoting impacts.

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Targeting mechanisms of resistance against anti-angiogenic cancer therapy and identification of biomarkers for therapeutic response

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Angiogenesis inhibition – the blockage of the growth of new blood vessels – has been approved for the clinical treatment of cancer patients. However, the patient responses are often at best transitory and the progression-free-survival only lasts a few months – also referred to as "resistance to anti-angiogenic therapy" (1). To identify potential mediators of resistance at the level of the target endothelial cells, mice bearing B16-F10 melanomas were treated for 24h with 60 mg/kg of the VEGF receptor kinase inhibitor sunitinib or vehicle control administered intraperitoneally. Blood vascular endothelial cells were sorted from these tumors by high-speed cell sorting, identified as CD45-/CD31+/podoplanin- cells. Using gene microarray analyses, we observed several molecular pathways related to angiogenesis down-regulated, whereas immune function-related pathways were up-regulated after treatment. Previously described potential resistance factors such as G-CSF were also up-regulated. Importantly, the HGF-receptor c- Met was 4.8-fold up-regulated in blood endothelial cells under treatment. Since HGF is known to promote angiogenesis, we are currently investigating whether combined treatment with sunitinib and a c-met inhibitor will improve the therapeutic outcome. Interestingly, we found several genes modulated associated with vascular permeability, including claudin-5, a tight junction molecule and permeability gene and VE-cadherin at the RNA and/or protein level. Using newly developed techniques for the non-invasive *in vivo* quantification of vascular permeability, we found a decrease of vascular leakage in tumors after 24h of sunitinib treatment. Importantly, reduced vascular leakage was also observed in the normal skin of sunitinib-treated mice, indicating that measurement of skin vascular permeability might serve as a new biomarker for anti-angiogenic response.

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Redirecting CD8 T lymphocytes against the cancer testis: NY-ESO-1 tumor antigen to potentiate immunotherapy of cancer

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Anti-self/tumor T cell function can be improved by increasing TCR-pMHC affinity within physiological limits, but paradoxically further increases (KD < 1μM) lead to drastic functional declines (1). Using human CD8+ T cells engineered with TCRs of incremental affinity for the tumor antigen HLA-A2/NY-ESO-1, we investigated the molecular mechanisms underlying this high affinity-associated loss of function. As compared with cells expressing TCR affinities generating optimal function (KD; 5μM to 1 μM), those with supraphysiological affinity (KD; 1 μM to 15 nM) showed impaired gene expression, signaling and surface expression of activatory/costimulatory receptors. Preferential expression of the inhibitory receptor PD-1 was limited to T cells with the highest TCR affinity, correlating with full functional recovery upon PD-L1 blockade. In contrast, upregulation of the phosphatase SHP-1 was broad, with gradually enhanced expression in CD8+ T cells with increasing TCR affinities. Consequently, pharmacological inhibition of SHP-1 with sodium stibogluconate augmented the function of all engineered T cells, and this correlated with the TCR affinity-dependent levels of SHP-1. These data highlight an unexpected and global role of SHP-1 in regulating CD8+ T cell activation and responsiveness, and support the development of therapies inhibiting protein tyrosine phosphatases to enhance T cell mediated immunity.

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Functional characterization of CD70, a biomarker specific for clear cell renal cell carcinoma

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CD70, a member of the tumor necrosis factor ligand superfamily, was previously identified as a specific biomarker for clear cell renal cell carcinoma (ccRCC). However, functional studies characterizing CD70 in ccRCC are lacking. Using a tissue microarray (TMA), high CD70 expression was observed in 78% of ccRCC. Microarray analysis confirmed the tissue microarray result. Notably, CD70 is expressed in all analyzed brain metastases originating from CD70 positive primary ccRCC. By analyzing the expression pattern of the von-Hippel-Lindau tumor suppressor (pVHL) and its target proteins, as well as the VHL mutation status, we found that CD70 is linked to the inactivation of pVHL. Re-expression of pVHL in the VHL-deficient cell lines leads to attenuated CD70 expression. It was reported that demethylation of CD70 promotor contributes to CD70 overexpression in various CD4+ T cells affecting immune diseases. To analyze the methylation status of the CD70 promoter in ccRCC we performed bisulfite sequencing. Interestingly, hypomethylation of the CD70 promotor was linked to overexpression of CD70 in ccRCC cell lines and tumor tissue. We identified ccRCC which co-expressed both the receptor of CD70, CD27. Only CD27 positive but CD70 negative ccRCC were associated with early tumor stage, low grade and better overall survival. Our data suggest that the upregulation of CD70 is characteristic feature for ccRCC. The expression of CD70 seems to be promoted by both the loss of pVHL and by promoter hypomethylation. Notably, the expression of the ligand CD70 and its receptor CD27 suggests its importance for the biology of this tumor subtype.

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Regulation of the human MutY homolog DNA Glycosylase (MUTYH)

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A high level of reactive oxygen species can lead to oxidative stress in human and animal cells, which has been shown to cause the majority of spontaneous DNA damage. One of the best characterized lesions of DNA is the formation of 7,8-dihydro-8-oxoguanine. Opposite this lesion, an incorrect A is frequently incorporated by replicative DNA polymerases, which, if not repaired, subsequently leads to G:C to T:A transversion mutation. This transversion is the most prevalent somatic variation occurring in different types of human cancers (1). To counteract this, the base-excision-repair pathway is initiated by action of the DNA glycosylase MUTYH, which excises mispaired A opposite the lesion. Germline mutations in MUTYH are correlated with a predisposition for the development of MUTYH-associated polyposis. Although an important role for MUTYH in cancer development is already demonstrated, very little is known about its regulation and posttranslational modifications. Due to the fact that different members of the base-excision-repair complex were shown to be regulated by the E3-ligase Mule (2,3), we sought to prove whether it also plays a role for the degradation of MUTYH. Our data show that MUTYH serves as substrate for Mcl-1 E3 ligase in an *in vitro* ubiquitination assay and that MUTYH is regulated by Mcl-1 E3 ligase *in vivo*. Further investigation will show the functional relevance of this regulation.

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RASSF1A loss deregulates hippo pathway signalling in tumourigenesis

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The tumour suppressor gene RASSF1A is inactivated through point mutation or promoter hypermethylation in the majority of human cancers. RASSF1A silencing is the most widely reported epigenetic event in sporadic human malignancies and has increasingly prognostic value, e.g. in lung, breast and bladder cancer. We have been focusing on the consequence of RASSF1A loss to tumour initiation and radiation sensitivity. We performed a genetic screen in mice using Sleeping Beauty (SB) transposon-mediated insertional mutagenesis, to identify candidate genes associated with tumourigenesis in the absence of Rassf1a. From the resulting leukaemias/lymphomas that developed in these mice, we identified 10 genes potentially associated with tumour formation in the absence of Rassf1a. We further analysed the synergy of the 2 hit model of tumour suppression by monitoring biochemical pathway activation and cellular proliferation assays. One of the top hits in our screen was the terminal differentiation transcription factor Runx2. Here, we describe how loss of RASSF1A promotes oncogenic YAP1-TEAD complexes and how RUNX2, p73 and TEAD effectively compete for YAP1 association. Together loss of both RASSF1A and RUNX2 exacerbate YAP1-TEAD levels consistent with the multi-step nature of cancer. We show that RUNX2 expression is frequently down-regulated in various cancers associated with poorer survival in patients with diffuse large B-cell lymphomas. Interestingly, concordant decreased expression levels of RASSF1 and RUNX2 were observed in both precursor T-cell acute lymphoblastic leukaemia and colorectal cancer, further supporting the hypothesis that dual regulation of YAP1-TEAD promotes oncogenic activity, thus providing a novel mechanism for RASSF1A-mediated tumour suppression.

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How can the combined action of faithful repair mechanisms (mismatch repair and base excision repair) generate DNA double strand breaks to induce class switch recombination?

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During class switch recombination (CSR), antigen-stimulated B-cells rearrange their immunoglobulin constant heavy chain (CH) loci to generate antibodies with different effector functions. CSR malfunction is linked to a rare primary immunodeficiency (1/100000 births), also referred to as Hyper-IgM syndrome, characterized by normal or elevated IgM levels and a decrease or complete absence of IgG, IgA and IgE, rendering the patients very susceptible to bacterial infections, especially of the upper respiratory tract. Search for the underlying molecular defect in this syndrome, both in patients and in mouse models, identified mutations in the activation-induced deaminase (AID) gene, as well as in genes encoding proteins involved in base excision repair and mismatch repair (MMR) (1,2). AID generates numerous U/G mispairs in the CH loci by converting cytosines to uracils. U/Gs are generally efficiently repaired to C/Gs by uracil DNA glycosylase-initiated base excision repair. Puzzlingly, U/G processing in CH loci of activated B-cells gives rise also to double strand breaks (DSBs), which subsequently trigger CSR. In an attempt to elucidate the molecular mechanism of CSR, we studied the processing of uracil-containing DNA substrates in extracts of MMR-proficient and -deficient human cells, as well as in a reconstituted system. We now show that the presence of uracils in opposite DNA strands (or of uracil in one strand and a strand break in the other) gives rise to MMR-dependent DSBs *in vitro* and to deletions *in vivo*. We posit that the DSBs arise through collision of MMR-catalyzed degradation tracts initiating at sites of uracil processing and that the deletions arise during the repair of these DSBs.

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The role of NEMO in renal cell cancer – towards a novel HIFα activation mechanism

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In clear cell renal cell carcinoma (ccRCC) the loss of functional von Hippel-Lindau protein (pVHL), correlated with upregulation of the nuclear factor-kappa B (NF-κB), leads to the stabilization of hypoxia inducible factor (HIF). A previous study with HEK293T cells showed a direct interaction between HIF2α and NEMO (the IκB-kinase-subunit NF-κB essential modulator), a member of the NF-κB pathway. However, the relevance of interaction between NEMO and HIFα and its dependency on pVHL in ccRCC is unknown. In this study we examined the influence of NEMO on HIFα and its biological significance for ccRCC. NEMO stabilizes HIF1α and of HIF2α independently of pVHL under both hypoxic and normoxic conditions, acting as their upstream regulator. The direct binding of NEMO to HIFα was also confirmed by coimmunoprecipitation. RCC tissue microarray analysis demonstrated correlation of NEMO expression with HIFα activation and its targets. Moreover we found that NEMO expression is directly correlated with wild-type pVHL and negatively correlated with mutated VHL. Furthermore, there was a significant association of NEMO expression with nuclear differentiation grade, as confirmed by stronger NEMO immunostaining observed in metastatic tissues compared to the primary tumors. Our results implicate a direct connection between NEMO and HIF-driven pathways in renal cell cancer, revealing additional mechanism of HIFα stabilization in the presence of functional pVHL. In light of personalized therapy strategies it will be crucial to investigate the exact mechanism(s) of NEMO regulation and its interaction with HIF in ccRCC as well as in other tumors.

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Targeting of thymosin beta4 gene expression decreases stemness and invasiveness in glioblastoma

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Thymosin β4 (TB4) is a pleiotropic actin-sequestering polypeptide that is involved in wound healing and developmental processes. TB4 gene silencing promotes differentiation of neural progenitor cells (1) whereas TB4 overexpression initiates cortical folding of developing brain hemispheres (2). However, a role of TB4 in malignant gliomas has not yet been investigated. We first analyzed TB4 expression on tissue microarrays and performed a REMBRANDT database interrogation. TB4 expression increased with the grade of malignancy in gliomas and was correlated with patient survival. *In vitro*, TB4 gene silencing by lentiviral transduction decreased migration, invasion, growth and self-renewal, and promoted differentiation and the susceptibility to undergo apoptotic cell death upon nutrient depletion in LNT-229, U87MG and the glioma stem-cell line GS2, respectively. *In vivo*, survival of nude mice bearing tumors derived from TB4-depleted glioma cells was improved and the tumorigenicity of the GS2 glioma stem-cell line was decreased. A genome-wide RNA expression array was performed to unravel the underlying molecular network mediating these effects. The gene expression pattern was shifted from the mesenchymal towards the pro-neural gene signature upon TB4 gene silencing, and clustering of differentially regulated genes involved TGF-β and p53 signaling networks. We conclude that TB4 is a key regulator of malignancy in glioblastoma that should be explored as a novel molecular target for anti-glioma therapy.

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Obesity-associated inflammation promotes liver carcinogenesis via Mcl-1 stabilization independent of IL-6 signaling

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Obesity increases the incidence of hepatocellular carcinoma development in part through activation of obesity-associated pro-inflammatory signaling. In lean mice, abrogation of IL-6Rα signaling protects against dietylnitrosamine-induced hepatocellular carcinoma development. Hepatocellular carcinoma protection occurs via Mcl-1 destabilization, thus promoting hepatocyte apoptosis. IL-6 regulates Mcl-1 stability via inhibition of PP1α expression promoting GSK-3β inactivation. In addition, IL-6 suppresses expression of the Mcl-1 E3 ligase (Mule). Consequently, IL-6Rα deficiency activates PP1α and Mule expression resulting in increased Mcl-1 turnover and protection against hepatocellular carcinoma development. In contrast in obesity, inhibition of PP1-α and Mule expression leading to Mcl-1 stabilization occurs independent from IL-6 signaling. Collectively, this study provides evidence that obesity-associated inflammation inhibits hepatocyte apoptosis through Mcl-1 stabilization independent from IL-6 signaling thus, promoting liver carcinogenesis.

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CD44s is required for epithelial to mesenchymal transition and stemness properties via ZEB1 activation

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Invasion and metastasis of carcinomas are often promoted by the aberrant epithelial to mesenchymal transition program at the invasive front of the tumor. The transcription factor ZEB1 is a crucial promoter of metastasis formation by inducing epithelial to mesenchymal transition and tumor initiating capacity. CD44, a cell surface protein, is used as marker to identify tumor initiating cells in many cancer types. It is involved in cell-cell interactions, cell adhesion and migration. Through alternative splicing, controlled by the epithelial splicing factor ESRP1, CD44 is differentially regulated during epithelial to mesenchymal transition, resulting in a switch from the variable exon-containing CD44v isoforms to the standard isoform CD44s, which is devoid of all CD44 variable exons. We examine the reciprocal interaction of CD44s and ZEB1 in their function as tumor initiation markers. CD44s and ZEB1 are co-expressed in poorly differentiated human cancer cells. Transient down regulation of CD44s results in decrease of ZEB1, which in turn leads to reduced sphere formation ability. Likewise, inhibition of CD44s increases ESRP1. Knockdown of ESRP1 decreases expression of CD44v isoforms, increases CD44s and ZEB1 and the sphere forming capacity. Additional knockdown of CD44s reduces the sphere formation. Furthermore, ZEB1 inhibits ESRP1 expression, which leads to upregulation of CD44s. In summary, we identified a positive feedback loop maintaining stemness and mesenchymal features in cancer cells: CD44s activates expression of ZEB1, which suppresses transcription of the splicing factor ESRP1, resulting in further increase in CD44s expression.

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Radioimmunotherapy and adjuvant radiosensitizing agents for combination therapy against ovarian carcinomas

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Front line therapies against advanced ovarian cancer, including surgery followed by a paclitaxel-platinum treatment, fail to result in a cure of disease. Apart from standard treatment procedures, radioimmunotherapy is a potential adjuvant treatment strategy since it has initially proved to be highly effective in patients with lymphomas. Although encouraging results have been achieved in hematopoietic malignancies, beneficial outcomes in patients with solid tumors are more limited due to increased radioresistance. In order to further enhance the radioimmunotherapy treatment efficacy against small solid ovarian tumor nodules including metastases, we investigate combination therapies *in vitro* and *in vivo* of radioimmunotherapy combined with adjuvant radiosensitizing treatments like taxanes [1].We use a radioimmunoconjugate derived from the tumorspecific monoclonal antibody chCE7 labeled with beta-particle emitting 177Lu for radioimmunotherapy. The antibody chCE7 is directed against the L1 cell adhesion molecule, a protein found in the nervous system that is also highly expressed in numerous tumors such as neuroblastoma, colon carcinoma, melanoma and ovarian carcinoma [2].Previous work from our laboratory has demonstrated that 177Lu-chCE7 is highly potent in treatment of disseminated ovarian cancer *in vivo* [3]. Based on this work it could be demonstrated that a combination therapy with paclitaxel can further increase the treatment efficacy *in vitro*. Data of induced G2/M phase cell cycle arrest and apoptosis upon different combination treatment conditions will be collated to investigate distinct treatment schedules for combined applications of radioimmunotherapy and chemotherapeutics *in vitro* and *in vivo*.

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HOTTIP and HOXA13 expression levels predict patients' survival and metastasis formation In hepatocellular carcinoma

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The HOX genes transcriptional factors family has been recently linked to the genesis and evolution of liver cancer. We observed that among the HOX genes, HOXA13 is highly deregulated in hepatocellular carcinoma (HCC) (1). More recently, a lncRNA located at the 5' end of the HOXA locus (in physical contiguity with HOXA13), named HOTTIP, has been identified. HOTTIP binds the WDR5/MLL complexes driving gene transcription along the entire HOXA locus (2). In this study we aimed to evaluate the impact of HOTTIP and HOXA13 deregulation on HCC pathogenesis. Total RNA extracted from 60-paired biopsies obtained from HCC patients w1as used to quantify HOTTIP/HOXA13 expression levels via qRT-PCR and subjected to global transcriptome analysis. HOTTIP/HOXA13 expression levels have been correlated with patients' clinico-pathological data. The qRT-PCR data confirmed that HOXA13 is highly deregulated in HCC with no major alteration found in non HCC-conditions. Furthermore, we outlined that HOTTIP is also deregulated in HCC but not in non HCC-conditions and that its expression directly correlates with HOXA13 levels. In addition, we found that both HOTTIP and HOXA13 expression levels predict patients' overall survival as well as metastasis formation. Finally, the global transcriptome analysis revealed that HOTTIP/HOXA13 overexpression in HCC identifies a specific subset of genes mostly involved in mRNA processing. siRNA against HOTTIP/HOXA13 *in vitro* experiments using the hepatoma cell lines HuH6 and HuH7, revealed that the knock down of these genes impaired cell proliferation and migration ability and further validated the strong interplay between them. Hence, we demonstrated for the first time that HOTTIP expression directly correlates with HOXA13 levels in HCC. Moreover, we reported that HOTTIP/HOXA13 deregulation as a key feature in HCC. Finally, we outlined HOTTIP/HOXA13 as predictive markers of HCC patients' outcome and metastasis formation.

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Identification of proteome alterations characteristic of the early stages of colorectal tumorigenesis

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Although a vast array of genomic and epigenomic changes related to colorectal tumorigenesis have been well detailed, the consequences of these alterations on the effectors of biological function (proteins) have not been comprehensively explored. In this project, we applied untargeted quantitative mass spectrometry and selected reaction monitoring techniques to analyze the proteome of precancerous colorectal adenomas. Total protein extracts were prepared from 30 paired adenoma/normal mucosa samples prospectively collected during colonoscopy, and five cancer (SW480, SW620, CACO2, HT29, CX1) and one non-cancerous (HCEC) colon epithelial cell lines. Following protein digestion, peptides were labeled with 8-plex isobaric tags and separated by OFFGEL electrophoresis. LC MS/MS was carried out in an LTQ Orbitrap Velos, and statistical methods were applied to perform differential analysis on protein expression. In tissues and cell lines, 3645 and 2019 unique proteins were identified and quantified, respectively. Multivariate analysis clearly distinguished adenoma from normal mucosa samples, and cancer cell lines from HCEC. Over 200 proteins displayed significant expression changes in adenoma (q-value<0.02, fold change=+/-1.5) in comparison with normal mucosa samples. One-third of these changes occurred presumably in the epithelial tumor component since they were similarly changed in our cancer/normal cell line model. More than 50% of proteins displayed expression changes similar to those of the corresponding mRNAs (our previous studies). Pearson's correlation: 3645 proteins, r=0.53; and 219 deregulated proteins, r=0.7. Our shotgun proteomic approach quantified over 3000 proteins in colorectal tissues that are expressed over four-fold order of magnitude. Some of the proteins found deregulated in adenomas are the subject of further investigation to identify putative diagnostic markers or therapeutic targets.

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Modulation of tyrosine kinase receptor activity by T-cadherin in cancer

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Cadherins are a superfamily of cell adhesion molecules that mediate calcium-dependent homophilic binding. T-cadherin is a unique cadherin because it lacks transmembrane and cytoplasmic domains and is membrane-anchored via a glycosylphosphatidylinositol moiety. Dysregulation of some cadherins, including T-cadherin, occurs in many human cancers. Histology of human squamous cell carcinoma (SCC) tissue reveals associations between T-cadherin expression levels and cell differentiation. Well differentiated specimens retain T-cadherin expression, while its loss is associated with tumor invasion. The function of T-cadherin in tumorigenesis is poorly understood. We aimed to clarify the role of T-cadherin in epithelial cancers. SCC cells were stably transduced (lentivector) with respect to T-cadherin silencing or overexpression. Functional and signaling characteristics were examined.

- T-cadherin silencing/overexpression enhances/decreases cell elongation and proliferation.
- T-cadherin silencing/overexpression enhances/decreases EGF-induced cell retraction and motility.
- T-cadherin expression levels influence EGF-induced changes in activities of Rho small GTPases: T-cadherin silencing/overexpression decreases/increases activation of Rac1 and Cdc42. RhoA activation is conversely regulated by T-cadherin expression levels.

- T-cadherin silencing releases EGFR from lipid rafts, rendering EGFR more accessible to EGF binding, thereby increasing EGFR pathway activity.
- T-cadherin silencing in other carcinoma cell types including colon and prostate adenocarcinoma cells modulates EGFR and IGF-1R activities and their cross-talk.

We conclude that T-cadherin acts as a \"negative\" regulator of EGFR pathway activity in SCC. T-cadherin modulates EGFR and IGFR pathway activities in colon and prostate adenocarcinomas. Auxiliary regulation of growth factor receptor activity and cross-talk by T-cadherin might represent a general mechanism underlying T-cadherin-dependent control of epithelial carcinoma behavior.

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DNA methylation in melanoma phenotype switching

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Our laboratory recently described the phenotype switching model for melanoma progression(1,2). This model hypothesizes that melanoma cells drive disease progression by switching between phenotypes of proliferation and invasion. Microarray experiments have shown that specific gene expression patterns differentiate between proliferative and invasive melanoma cell phenotypes. *In vitro* the proliferative and invasive phenotypes are morphologically distinct and differ in proliferation rate and invasiveness. Phenotype switching may be an important process for establishing molecular heterogeneity in melanoma. To investigate the role of DNA methylation on melanoma heterogeneity we performed DNA methylation profiling of 5 proliferative and 5 invasive melanoma cell cultures by methylated DNA immunoprecipitation (MeDIP) microarray analysis. The proliferative and invasive phenotypes exhibited distinct methylation profiles. Combined analysis of gene expression and MeDIP datasets for differentially methylated and expressed genes between the proliferative and invasive phenotype revealed enrichment for genes involved in neural crest differentiation, such as SOX9 and FGF2. These results suggest that DNA methylation might be a mechanism which regulates neural crest differentiation factors in melanoma and reexpression of these neural crest differentiation factors in the invasive phenotype suggests that the melanoma cells return to a dedifferentiated state for invasion.

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The impact of CD27 signaling on Wnt-mediated T cell expansion

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The costimulatory molecule CD27 and its unique ligand CD70 are notably involved in adaptive immunity. CD27 belongs to the tumor necrosis factor receptor (TNFR) family and is expressed on human and murine T, B and NK cells and hematopoietic stem cells. CD70 expression is restricted to activated lymphocytes and mature dendritic cells upon immune activation. Triggering of CD27 on T cells promotes T cell survival, expansion and differentiation(1). Whereas CD27-mediated T cell survival mechanisms, which include an autocrine IL-2 feedback loop, have been studied for some time now, the way by which CD27 stimulation increases T cell proliferation is less well understood. We recently demonstrated that the CD70-CD27 interaction stimulates leukemia stem cell proliferation by increasing Wnt signaling. Intracellularly, CD27 promoted nuclear localization of β-catenin via TNFR-associated factor 2 (TRAF2) and the TRAF2 and NCK-interacting protein kinase (TNIK)(2). We now hypothesized that CD27 may have an impact on T cell expansion via the Wnt pathway as well. To investigate a possible link between CD27 and Wnt signaling in T cells, we activated human CD8+ T cells in the presence or absence of an inhibitory anti-CD27 monoclonal antibody *in vitro*. CD27 stimulation activated Wnt target gene transcription and increased nuclear localization of TNIK and β-catenin. We propose that CD27 signaling promotes T cell proliferation by activating the Wnt pathway via TRAF2 and TNIK. Targeting the CD27-TRAF2-TNIK-β-catenin signaling axis may offer an attractive strategy to modulate CD8+ T cell responses in infections and autoimmunity.

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Mouse models of autochthonous cancer to study local immune subversion

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The tumor environment delivers suppressive signals to the immune system, and these signals impair the effector function of tumor-infiltrating lymphocytes. The identification of these signals, their targets and their mode of action, however, largely remain issues to be resolved. We used transgenic adenocarcinoma of mouse prostate (TRAMP) animals, a well-known autochthonous murine model for prostate cancer induced by tissue specific SV40 large T expression, to study the interaction of the adaptive immune system and cancer cells in order to investigate which cell types and molecules are crucial in deflecting immune surveillance. We analyzed the tumor-specific T cell response in the spleen, the draining nodes and in the tumor of TRAMP mice. When transferring TCR transgenic CD8 T cells specific for epitope I of SV40LT, we observed rapid local and peripheral immune subversion of adoptively transferred cells in tumor-bearing animals. Homing and proliferation were not affected, but effector functions of these cells were greatly impaired. When using a combination of blocking antibodies for co-inhibitory molecules (PD-1, CTLA-4 and TIM-3), we could restore to some extent the function of transferred cells in the periphery, but not at the tumor site. At the same time, we are in the process of generating an inducible CreERT/LoxP-based model to express the immunologically well-characterized oncogene SV40LT with the luciferase in a tissue specific fashion. This model will allow the sensitive detection of arising tumors and permit precise dissection of tumor-specific immunity over time. By comparing fundamentally different target tissues, we may find mechanisms that are active in every malignancy and some that are organ-specific.

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Self-renewing cellular compartments in embryonal rhabdomyosarcoma are modulated by hedgehog signaling

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Rhabdomyosarcoma (RMS) is a heterogeneous group of malignancies with features of impaired skeletal muscle differentiation and represents the majority of the soft tissue cancers in the pediatric age group. Current treatment regimens offer some clinical respite but with a significant risk of adverse side effects and risk of secondary tumors later in life. Identifying and targeting sub-populations of tumor cells with higher tumorigenic potential and self renewal capacity has the capacity to not only decrease tumor burden but also to lower the probability of relapse. Using the sphere formation assay, we could select for self-renewing cells from the heterogeneous monolayer cultures of the embryonal RMS (eRMS) cell lines, RD and Rh36, these populations having multipotent differentiation ability *in vitro* and greater tumorigenic potential *in vivo* [1]. These cells also showed increased expression of embryonic stem cell genes. Interestingly, quantitative gene expression analysis revealed increased expression of various components of the Hedgehog pathway, a developmental pathway important in eRMS biology. Inhibition or activation of the pathway *in vitro* by small molecule agents led to increased or decreased self renewing ability, respectively. Furthermore we observed that eRMS adherent monolayer cultures contained a sub-population of GLI1-positive cells which could be modulated by pathway manipulation. Importantly, inhibition of the hedgehog pathway *in vitro* led to significantly delayed tumor initiation *in vivo*. Ongoing experiments are intended to confirm these results at the genetic level. We postulate that the hedgehog pathway sustains a primitive cellular compartment necessary for tumor initiation within embryonal RMS cell lines.

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The impact of innate signals on tumor-specific immunity

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Toll-like receptors (TLRs) play key roles in immune responses against infection and are crucial targets of most adjuvants, however, our understanding of their role in cancer is nascent. TLR signaling depends on the adaptor molecule MyD88, except for TLR-3, which exclusively uses TRIF as adaptor protein, and TLR-4, which uses MyD88 and TRIF. Opposing roles of MyD88 have been described in tumor development. On one hand MyD88 is required for tumor initiation both *in vitro* and *in vivo* (1); on the other hand,absence of MyD88 induces pancreatic carcinogenesis via Th2 cells (2). Furthermore, a contribution of TLR-4 to tumor control upon treatment with particular chemotherapeutics has been shown (3). Because of evident discrepancy, we decided to explore the role of MyD88 signals on tumor-specific, adaptive immune responses. WT (C57BL/6) and MyD88-/- were injected s.c. with 2x105 B16gp, 1x106 MC-38 or 1x106 MC-38-OVA-GFPdim cells. Tumor growth was measured with a calliper and documented as length x width (surface, mm2). At the end of the experiment tumors were collected and flow-cytometric (FACS) analysis was performed to quantify accumulation of various immune cells. Irrespective of the tumor type, we observed significantly faster tumor growth in MyD88-/- mice than in WT mice. FACS analysis revealed a decreased accumulation of leukocytes (CD45.2+ cells) in MyD88-/- tumors when compared to WT tumors. Within the leukocyte subset, the percentage of infiltrating CD11b+ myeloid cells was significantly higher and the percentages of CD8+ T cells, CD4+ T cells and DCs were significantly lower in tumors in MyD88-/- mice. We conclude that the adaptor molecule MyD88 is crucial for the recruitment of immune cells within tumors, which helps in subsequent control of tumor growth.

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ATPase p97/VCP regulates the polyubiquitination of nucleotide excision repair proteins

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Long-term ultraviolet (UV) exposure causes an increasing number of skin cancer cases, particularly in the fair-skin population world-wide. UV irradiation induces harmful DNA lesions which can lead to deleterious mutations and ultimately to skin cancer. Nucleotide excision repair is the only DNA repair mechanism that removes UV-induced DNA lesions before their conversion into irreversible mutations and therefore represents a crucial DNA repair pathway to protect genomic stability. The nucleotide excision repair system is controlled by a precise regulation cascade in which post-translational modifications, especially polyubiquitination of several nucleotide excision repair proteins constitute an important signaling mechanism. Two initiator proteins of the global genome this pathway, DNA damage-binding protein 2 (DDB2) and xeroderma pigmentosum complementation group C (XPC) are polyubiquitinated upon UV irradiation and have a crucial role in DNA repair efficiency due to their UV lesion recognition activity. Interestingly, the purpose of polyubiquitination seems to vary between the two proteins: DDB2 is degraded upon polyubiquitination whereas the fate of polyubiquitinated XPC in the physiological environment of tightly packed chromatin remained enigmatic. The regulation of proteasomal protein degradation comprises a complex series of events in which several proteins are involved. Recently, a well-known regulator of the ubiquitin-proteasome system, ATPase p97/VCP, was identified as a new player in double-strand break repair (1). Here, we show that p97 also regulates the polyubiquitination and degradation of both XPC and DDB2. p97 is recruited to UV lesion sites quickly upon UV treatment and thus has an effect on recruitment and removal of NER proteins. p97-depleted cells show hyper-ubiquitination of XPC whereas the levels of DDB2 are stabilized upon UV treatment resulting in impaired UV lesion repair rates. These findings propose a novel role for p97 and a new exciting regulation mechanism for nucleotide excision repair in mammalian cells.

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Histone acetylation and DNA demethylation in cutaneous T cell lymphoma treatment

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Cutaneous T-cell lymphomas are a heterogeneous group of malignancies derived from skin-homing T cells. Cutaneous T-cell lymphomas is a chronic and at times debilitating disease with many unresolved issues regarding its pathogenesis and treatment. The current moderate success of histone deacetylase inhibitors in the clinical practice for treatment of this disease encourages the investigation of combinational therapy in order to increase the response rate(1). The combination treatment with Romidepsin and Azacytidine compared to single agent or untreated cells demonstrated a decrease in cell viability via MTT assays and increase in apoptosis and necrosis via flow cytometry with Annexin V staining. Increased expression of cell cycle regulators such as p15, p16, p21 as well as down-regulation of CDK4 following combination treatment than occurred in response to either drug alone suggests this combination treatment counteracts the loss of cell cycle control most efficiently in cutaneous T-cell lymphoma. This combination triggers an increase in cleaved caspase 9 as well as cleaved caspases 3 and 7 and cleavaged PARP1. We demonstrate a significant re-expression of RhoB in the combination treatment which was validated at an RNA and protein level. Defective regulation of apoptosis has been considered as a main cause for the accumulation of clonal T cells and therefore we hypothesize, based on our current results, that RhoB might have an important role in the sensitization of the tumor cells to DNA damage and subsequent apoptosis(2). Furthermore, peripheral blood lymphocytes from a SS patient were used to validate the results we attained *in vitro*.

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Activation of the lens epithelial-derived growth factor by human papillomaviruses

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Human papillomaviruses (HPVs) are closely associated with tumor development in humans. Oncogenic HPVs, most notably HPV16 or HPV18, cause cervical cancer, the second most common malignancy in females worldwide. The viral E6 and E7 oncoproteins play the key role for HPV-induced oncogenesis. The elucidation of the cellular pathways dysregulated by E6 and E7 is critical for understanding the molecular mechanisms of HPV-induced cell transformation [1]. In order to identify novel cellular pathways targeted by HPVs, a genome-wide transcriptome analysis upon siRNA-mediated inhibition of E6/E7 oncogene expression was performed in HPV-positive cancer cells. The lens epithelial-derived growth factor (LEDGF) gene was found to be significantly repressed, both at the RNA and at the protein level. This finding indicates that continuous viral oncogene expression is required to maintain intracellular LEDGF levels. Silencing of LEDGF expression strongly blocked the colony formation capacity of HPV-positive cancer cells. Thus, LEDGF stimulation by HPVs could be an important contributor to the growth and/or survival of HPV-positive cancer cells. In order to identify pathways which could mediate the growth-promoting effect of LEDGF, we performed transcriptome analyses upon LEDGF silencing and identified potential downstream target genes. Ongoing analyses concentrate on the further characterization of these target genes. In addition, and in view of its postulated role for DNA repair, we are currently investigating whether LEDGF activation by the HPV oncogenes contributes to the resistance of HPV-positive cells to chemo- and radiotherapy.

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CD70 reverse signaling on natural killer cells enhances tumor control

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CD70 is the unique ligand for the TNF-receptor superfamily member CD27.1 CD70 is not expressed in healthy individuals. Upon immune activation CD70 is transiently induced on B- and T lymphocytes, natural killer (NK) cells and subsets of dendritic cells.2,3 The CD27-CD70 interaction provides a positive costimulatory signal through various downstream pathways resulting in proliferation and survival of lymphocytes, as well as differentiation towards effector cells. Recently, we could demonstrate that CD27 signaling increases the frequency of intratumoral regulatory T cells and promotes growth of solid tumors. As for other TNF family members, CD70 reverse signaling has been reported on normal and malignant B cells, T cells and NK cells. 2 However, the physiological relevance of CD70 reverse signaling *in vivo* is poorly understood. In the present study, we analysed the role of CD70 reverse signaling in the immunological of control of solid tumors. Tumor fragments expressing a truncated CD27 receptor consisting of the extracellular domain were transplanted subcutanteously on both flanks of BL/6 mice and tumor growth was monitored. Growth of tumors expressing the truncated form of CD27 was significantly reduced when compared to growth of parental tumor cells. Furthermore, tumor growth of cells expressing truncated CD27 was restored to growth of non-transfected control cells in mice treated with a monoclonal antibody blocking the CD27-CD70 interaction. In contrast, tumor growth of cells expressing truncated CD27 was significantly slower in Rag2-/- mice, indicating that inhibition of tumor development was independent of adaptive immunity. Depletion of NK cells in RAG-/- restored growth of tumors expressing truncated CD27 to growth kinetics of control cells. Taken together, CD70 reverse signaling on NK cells contributes to tumor control.

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Protein kinase C signaling prevents irradiation-induced apoptosis of primary human fibroblasts

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Primary cells respond to irradiation by activation of the DNA damage response, cell cycle arrest and eventually senescence or apoptosis. It is not clear, what pathway regulates the induction of either apoptosis or senescence. Primary human fibroblasts are able to withstand high doses of irradiation and to prevent irradiation-induced apoptosis. However, the underlying regulatory basis for this phenotype is not well understood. Here, a kinetic network analysis based on reverse phase protein arrays in combination with extensive western blot and cell culture analyses was employed to decipher the cytoplasmic and nuclear signaling networks and to identify possible anti-apoptotic pathways. This analysis identified activation of known DNA damage response pathways (e.g., ATM-dependent phosphorylation of p38 and Hsp27, p53 and Chk1) as well as of pro-survival (e.g., MEK-ERK, CREB) and anti-apoptotic markers (e.g., Bad, Bcl-2 and PKC). Interestingly, protein kinase C family members were activated early upon irradiation, suggesting a regulatory function in the irradiation-response of these cells.
Inhibition or down-regulation of protein kinase C in primary human fibroblasts caused irradiation-dependent down-regulation of the identified pro-survival (CREB phosphorylation) and anti-apoptotic (Bad phosphorylation, Bcl-2) markers and thus lead to a proliferation stop and to apoptosis. Our analysis suggests that cytoplasmic protein kinase C signaling conditions irradiation-stressed MRC-5 and IMR-90 cells by allowing DNA repair and preventing apoptosis. These findings contribute to the understanding of the cellular and nuclear irradiation-response and may thus eventually improve the efficacy of radiotherapy and help overcome tumor radioresistance.
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Malignant lymphoma B cells induce dysfunctional cytotoxic T cells

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B cell non-Hodgkin lymphoma is the fifth most common cancer in humans. As in several types of cancers, cytotoxic T lymphocytes are found to be dysfunctional. However, since little is known about how malignant cells modulate the effector function of cytotoxic T lymphocytes in haematologic malignancies, we aimed to answer this question in a murine model of non-Hodgkin lymphoma. To this end, we used Eu-myc transgenic mice that constitutively express the myc oncogene in the immunoglobulin gene locus under the potent Igh enhancer (Eu) and spontaneously develop aggressive B cell lymphoma. We first investigated the expression of three inhibitory receptors and absolute T cell numbers in lymphoid organs. We found significantly reduced numbers of CD8+ and CD4+ T cells in lymphoma-bearing mice when compared to controls. A large proportion of the remaining T cells expressed PD-1, 2B4 and Lag-3. Furthermore, host T cells and adoptively transferred naïve CD8+ TCR transgenic (p14) T cells specific for the gp-33 antigen of lymphocytic choriomeningitis virus (LCMV) exhibited limited cytokine production and killing capacities after immunisation with antigen in lymphoma bearing mice. The impaired cytotoxic T lymphocytes function was not tumor antigen-specific and could be reversed after transfer into secondary healthy recipients. Co-culture and transwell experiments revealed that lymphoma B cells directly inhibited T cell function by the release of a soluble factor <1kDa. Our data indicate that lymphoma B cells induce cytotoxic T lymphocytes dysfunction by the release of a small soluble metabolite. Its identification may provide a new therapeutic strategy to enhance immunogenicity against lymphoma.

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Ribonucleotide monophosphates amongst us: ribonuclease H2-mediated removal of ribonucleotides as a strand-discrimination signal for eukaryotic mismatch repair

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To improve replication fidelity, mismatch repair (MMR) must detect non-Watson-Crick base pairs and direct their repair to the nascent DNA strand. How eukaryotic MMR identifies the newly-synthesized strand, however, has remained enigmatic. Because mammalian MMR *in vitro* requires strand discontinuities for initiation, MMR *in vivo* has been postulated to initiate at Okazaki fragment termini in the lagging strand, and at nicks introduced into the leading strand by the mismatch-activated endonuclease of PMS2. We now show that a single ribonucleotide in the vicinity of a mismatch can act as a strand discrimination signal in human cell extracts and that MMR activation in this system is dependent on a ribonuclease RNase H2. Coupled with the fact that loss of RNase H2 in S. cerevisiae leads to increased mutagenesis caused by a mild defect in MMR, we postulate that MMR *in vivo* might also initiate at nicks generated by RNase H2 during the removal of ribonucleotides misincorporated into newly-synthesized DNA during replication.

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IL6 deficiency leads to reduced liver damage and tumorigenesis in Mcl-1Δhep mice

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Many human chronic liver diseases (e.g. viral hepatitis) are characterized by increased (apoptotic) cell death and proliferation of hepatocytes. Mcl-1Δhep mice, mice with a hepatocyte-specific knock-out of the anti-apoptotic Bcl-2 protein Mcl-1, show chronic loss of hepatocytes accompanied by compensatory hyper-proliferation, finally leading to hepatocarcinogenesis and thus are a suitable model for studying human hepatocarcinogenesis. The pro-proliferative cytokine Il6 is well known as a key driver of liver tissue regeneration and overexpressed in hepatocellular carcinoma tissue. By utilizing the Mcl-1Δhep mouse model we are able to uncover the role of IL6 in hepatocarcinogenesis. For this investigation, Mcl-1Δhep mice were backcrossed to mice deficient of IL6 (IL6-/-) and analysed at 2 and 12 months of age. By analysing liver damage in Mcl-1Δhep-IL6-/- mice at 2 months of age, we observed a significant reduction of liver cell damage (reflected by aminotransferase levels). In addition, histological and morphological analyses revealed a significant decrease of apoptotic and proliferative hepatocytes. Secondly, Mcl-1Δhep-IL6-/- mice developed significantly fewer liver tumors compared to Mcl-1Δhep mice at 12 months of age. Although the remaining hetapocellular carcinoma in Mcl-1Δhep-IL6-/- mice did not did not exhibit different morphology, they displayed an altered molecular signature. We conclude that Il6 deficiency partially rescues liver damage and tumorigenesis in the Mcl-1Δhep mouse model. The remaining hepatocellular carcinomas in Mcl-1Δhep-IL6-/- mice might represent the human situation of anti-IL6 therapy resistant tumors and therefore enable to uncover molecular escape mechanisms. Our findings illustrate the intimate interplay of cell death and regeneration in hepatocarcinogenesis.

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Human papillomavirus (HPV) E6/E7 oncogenes affect both the contents and amounts of exosomes released from HPV-positive cancer cells

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The human papillomavirus (HPV) E6/E7 oncogenes play a crucial role for HPV-induced carcinogenesis. Exosomes are small vesicles of endosomal origin that are secreted by many cells, including tumor cells. Here, we investigated whether HPV E6/E7 expression may influence the contents or amounts of exosomes released from HPV-positive cancer cells [1]. We found that exosomes secreted from HeLa cells are enriched for survivin protein. RNA interference studies revealed that maintenance of both intracellular and exosomal survivin amounts was strongly dependent on continuous E6/E7 expression. This indicates that intracellular HPV activities are translated into visible alterations of exosomal protein contents. Besides survivin, HeLa exosomes contain additional members of the inhibitor of apoptosis protein (IAP) family (XIAP, c-IAP1, livin). In contrast, we did not obtain evidence for an exosomal location of the HPV E6 and E7 oncoproteins. Moreover, we found that silencing of HPV E6/E7 expression led to a significant increase in exosome amounts released from HeLa cells. This effect was associated with the reinduction of p53 and stimulation of the p53 target genes TSAP6 and CHMP4C which can enhance exosome production. Taken together, our results show that the HPV E6/E7 oncogenes can profoundly affect both the composition and amounts of exosomes secreted by HPV-positive cancer cells. This indicates that HPVs can induce molecular signatures in exosomes which may affect intercellular communication and could be explored for diagnostic purposes.

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Silencing c-Myc transcription with promoter-targeting small interfering RNA inhibits prostate cancer stem cell expansion and tumorigenicity

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There is increasing evidence that prostate cancer, like other cancers, is driven by a rare population of cancer cells that display stem-like properties. Several studies link therapy resistance, disease progression and recurrence to these cancer stem-like cells (CSCs) within the heterogeneous tumor cell population. c-Myc is a transcription factor that plays an important role in human cancers affecting cell proliferation, survival and metabolic adaptation. c-Myc has also a central function in stem cell biology and therefore may represent an attractive target to develop CSC-specific therapies. We have shown previously that c-Myc transcription can be effectively silenced by a siRNA targeting a promoter-associated noncoding RNA that we had identified in the c-Myc gene. Here, we investigated the effects of c-Myc transcriptional silencing on prostate CSC. Treatment with promoter-targeting siRNA reduced the fraction of CSCs both *in vitro* and *in vivo*. Combined analysis of senescence and cell surface markers showed that senescence occurred prevalently in the CD44+/CD24- cell subpopulation leading to impaired self renewal capability and reduced *in vivo* tumorigenicity and metastatic capability. Furthermore, systemic delivery of promoter-targeting siRNA reduced c-Myc expression and tumor growth in a mouse xenograft model. These results are consistent with the role of c-Myc in CSC maintenance and show that c-Myc silencing leads to senescence and loss of tumor-initiating capability of prostate CSCs. Our findings demonstrate also that an RNAi-based transcriptional therapy targeting a gene involved in the prostate CSC maintenance could be an effective approach to block tumor development and progression.

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In vivo profiling of DNA damage and repair kinetics after antineoplastic treatment: use of a minimally invasive approach

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Companion animals such as dogs frequently develop tumors with age and, similarly to human malignancies, displaying interpatient tumoral heterogeneity. Tumors are frequently characterized with regard to their mutation spectra and changes in gene expression or protein levels. Among others, these changes affect proteins involved in the DNA damage response (DDR), which was a basis for development of numerous clinically relevant cancer therapies. Though the effects of different DNA damaging agents, as well as DDR kinetics, have been well characterized in human cells *in vitro*, very little is known about the kinetics of DDR in tumor and normal tissues *in vivo* (1). This is mainly due to the fact that sampling of tissue is very difficult. In this work, the fine needle aspirates technique was chosen as a minimally invasive sampling method to examine how cells respond to DNA damaging agents *in vivo*. Tumor samples were repeatedly collected from canine patients before and several times after anti-neoplastic treatment, namely radiotherapy. Initial analysis of 60 samples revealed that 52 aspirates were of adequate quality. By using the alkaline comet assay, we showed a dose-dependent induction of DNA damage in different tumor samples after radiotherapy. Using a similar approach, we additionally detected changes in DNA repair kinetics between tumors, doses, and analyzed fractions. These initial findings suggest that fine needle aspirates, as a minimally invasive approach, may be used to reliably identify specific DDR defects in a clinical setting and could potentially contribute to better understanding of DNA repair processes in tumor and normal tissues after anti-neoplastic treatment.

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Mechanisms of collective invasion in human cancer patients

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Podoplanin, a small mucin-like protein, promotes tumor cell spreading, migration and invasion in both mouse models and human tumor samples. In human samples, podoplanin is co-expressed with E-cadherin in the invasive front. Moreover, in a Rip1Tag2 mouse model, podoplanin promotes invasion of β-cell tumors and bypasses epithelial-to-mesenchymal transition. When podoplanin-expressing human cancer cells are injected in an athymic mouse model, tumor cells rearrange into a podoplanin-expressing outer (collectively invasive) cell layer and a podoplanin-negative interior. An analysis by Affymetrix chip indicated a significant activation of interferon (IFN) signaling in these cells(1). In this project, we sought to determine if IFN signaling also plays a role during the invasive behaviour of human tumor cells generally. We analyzed freshly-frozen serial biopsies from lung and head-and-neck cancer patients before and after oncological treatment. From 65 tested tumors, 30 were podoplanin-positive: 13 of 15 from head and neck tumors, 5 of 26 from lung adenocarcinoma and 7 of 12 from squamous-cell lung tumors. Twenty tumors were double positive for podoplanin and for phosphorylated signal transducer and activator of transcription 1 or 3, which is activated by INF and other cytokines. In selected biopsies, the immunofluorescence status was confirmed by quantitative RT-PCR. Our future study will focus on enriching the content of podoplanin-positive cells by selecting invasive front areas in samples, and using laser capture microdissection to isolate them. This material will then be further analyzed using PCR arrays to investigate the role of IFN signaling in the invasive front.

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A new recurrent gain at locus 11q24.3 contributes to the pathogenesis of diffuse large B cell lymphoma (DLBCL) increasing the expression of ETS1 and FLI1 oncogenes.

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DLBCL develops from germinal center (GC) B cells, and contains two main subgroups: GCB-and ABC-DLBCL. Transcription factors controlling GC reactions, such as BCL6 and BLIMP1/PRDM1, are often deregulated by genomic aberrations. We sought to study the pathogenetic role of an 11q24.3 recurrent gain in DLBCL patients. Available clinical samples and cell lines were analysed as follows. Clinical samples by DNA-profiling (Affymetrix 250K SNP, 166 cases); cell lines by lentiviral infection, immunoblotting, real-time-PCR, proliferation assay, FACS, ChIP and RNA-profiling (Illumina-HumanHT-12v4 Expression-BeadChip). In 26% of DLBCL, 11q24.3 was detected with predominance in ABC-DLBCL ($P<0.05$) subtype. It was associated with high expression of the transcription factors ETS1 and FLI1 ($P<0.05$), which are recognized as oncogenes. To study the biological significance of 11q24.3, ETS1 and FLI1 were down-regulated in a DLBCL cell line bearing the gain (OCI-Ly7) and in other DLBCL cell lines. In OCI-Ly7 down-regulation causes cell death, in a cooperative manner, and also a decrease in cell proliferation, with a prominent role of FLI1. In the other cell lines, only FLI1 silencing caused cell mortality. Molecularly, down-regulation of ETS1 caused an up-regulation of BLIMP1, the master regulator of plasma cell differentiation. RNA-profiling analysis, after ETS1 and FLI1 silencing, highlighted an important role of both factors in different B cell developmental pathways and cell cycle regulation. We conclude that in DLBCL, a recurrent gain at 11q24.3 determines the over-expression of the transcription factors ETS1 and FLI1. Functional experiments suggest that these genes are essential for viability of DLBCL cells and that ETS1 contributes to the developmental arrest during GC B cell transition towards plasma cells.

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Genomic profiling of anaplastic large cell lymphoma

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Anaplastic large cell lymphoma (ALCL), one of the most common T-cell lymphomas, has been separated in two distinct subtypes wholly based on the presence or absence of translocations involving the ALK gene (1,2). However, cases that do not express ALK (30-40%) are still considered only a provisional entity and the relationship between these two subtypes remains unclear (3). To identify the genetic events underlying the pathogenesis and to recognize the characteristic lesions of ALK+ and ALK-, we studied 69 ALCL (31 ALK-, 33 ALK+ and 5 cALCL) using SNP-based arrays using GeneChip Human Mapping SNP 6.0 array (Affymetrix, Santa Clara, CA, USA). The genomic profiles revealed a higher number of lesions in ALK- cases compared to the ALK+ ones. The most common lesions were -6q21 e -17p13 (TP53). In particular, the 6q21 loss, more common among ALK- cases, affects PRDM1 gene coding for BLIMP1 protein. PRDM1 and TP53 inactivation were often concomitant and observed in more than half of ALK- cases. PRDM1 was

inactivated due to deletions, homozygous deletions or mutations in 39% of ALK- cases and only in 3% of ALK+ cases. Functional *in vitro* and *in vivo* experiments showed that the reintegration of BLIMP1 in cell lines with -6q21 determined an arrest of cell proliferation with increase of apoptosis and cell cycle arrest, suggesting that PRDM1 acts as a tumor suppressor gene in ALCL.

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Targeting of the oncogenic transcription factor PAX3/FKHR through inhibition of polo-like kinase 1

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Oncogene addiction is common to a broad spectrum of tumours and inhibition of oncogenes may help to develop alternative treatment strategies to improve cancer therapy. However, thus far it has not been possible to target oncogenic transcription factors as they usually do not respond to small-molecule inhibition. Alveolar rhabdomyosarcoma (aRMS) is an aggressive childhood tumour characterized by the expression of the tumour-specific transcription factor PAX3/FKHR. This transcription factor is essential for survival of tumour cells and its phosphorylation is required for efficient transcriptional activity (1). We hypothesized that identification of upstream protein kinases mediating PAX3/FKHR phosphorylation will be an efficient strategy to impair the activity of the oncogenic protein and subsequently induce tumour cell specific apoptosis. Having performed siRNA and kinase-inhibitor library screens against the human kinome, we suggested that polo-like kinase 1 (PLK1) represents an attractive therapeutic target. Its inhibition by RNAi and PLK1 inhibitors reduce PAX3/FKHR activity and consequently the viability of aRMS cells. Investigating the mechanisms of impaired PAX3/FKHR activity, we observed two ways of regulation. First, PAX3/FKHR protein stability is indirectly affected by cell cycle arrest upon PLK1 inhibition, whereas the second mechanism is independent of this arrest. We found a direct interaction of PAX3/FKHR and PLK1 suggesting phosphorylation of the transcription factor by PLK1. We are therefore analysing potential phosphorylation sites already detected by *in vitro* kinase assays and mass spectrometry. Most importantly for novel therapeutic approaches, PLK1 inhibitors caused tumour regression in our xenograft experiments. Our data suggest that PLK1 warrants further investigation as a novel drug target in aRMS and may lead to a general strategy for treatment of tumours addicted to oncogenic transcription factors.

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The ETS transcription factor ELF3 is involved in a positive feedback loop leading to constitutive NF-κB activation and prostate cancer progression

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Activation of inflammatory signaling occurs frequently in human cancers but the underlying mechanisms are often unclear (1). Here, we describe a novel mechanism linking the ETS transcription factor ELF3 with NF-κB activation and prostate cancer progression. We found that ELF3 is frequently overexpressed in human primary and metastatic tumors. In prostate cancer cells and tumor xenografts, ELF3 mediated key transforming phenotypes and transcriptional changes in relevant oncogenic pathways. We found that ELF3 was induced by IL-1β through NF-κB and acted as a crucial mediator of the protumorigenic effects and transcriptional changes induced by this cytokine in prostate cancer cells. ELF3 in turn contributed to both constitutive and IL-1β-induced activation of NF-κB by interacting with the NF-κB subunits p65 and p50, enhancing nuclear translocation and transcriptio-

nal activity of the NF-κB complex, and inducing p50 transcription. Bioinformatic analysis revealed that the ELF3, NF-kB and IL-1β gene signatures significantly converged in various inflammatory and experimental cancer models suggesting an active role of ELF3 in mediating these transcriptional programs. Consistently, gene expression profiling showed enrichment of NF-κB features in prostate cancer cell lines and human tumors with high level of ELF3. Moreover, upregulation of ELF3 and NF-κB coexisted frequently in human prostate tumors and was associated with significantly reduced overall survival and increased disease recurrence. Collectively, this study provides a mechanistic link between ELF3, activation of inflammatory signaling and prostate cancer progression and may open new avenues for patient stratification and design of context-dependent strategies for patients with clinically aggressive and high risk tumors.

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Furin: a novel therapeutic target in the paediatric soft tissue sarcoma rhabdomyosarcoma

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Our research focuses on the investigation of potential therapeutic targets in rhabdomyosarcoma (RMS). RMS is the most common soft tissue sarcoma in children and comprises two main paediatric subtypes: embryonal RMS (ERMS) and alveolar RMS (ARMS). The prognosis and survival rate is often very poor and therefore new therapy approaches are required.
Upon panning RMS cells of both subtypes with a phage-displayed cyclic random peptide library we identified the proprotein convertase furin as a promising target for targeting peptides enabling us to deliver chemotherapeutic drugs specifically to RMS cells. Further investigations revealed that furin, which we found to be the proprotein convertase with the highest expression level throughout different paediatric soft tissue sarcoma types, plays an important role in promoting migration and invasion of ERMS and ARMS cells *in vitro* as well as in enhancing tumour growth *in vivo*. Thus we hypothesis that enhanced RMS growth relies on furin cleavage of relevant substrates such as growth factor receptors (IGF-1Rβ) or angiogenic factors (VEGF-C). We have investigated the impact of overexpression, silencing and inhibition of furin on the processing of different substrates by Western blotting and found that indeed the abundance of mature IGF-1Rβ and VEGF-C depends on the level of active furin.

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JAK2/STAT5 inhibition circumvents resistance to PI3K/mTOR blockade: a rationale for cotargeting these pathways in metastatic breast cancer

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Hyperactive PI3K/mTOR signaling is prevalent in the majority of human malignancies (1) and its inhibition exhibits potent antitumor activity in a wide spectrum of solid cancers. Unfortunately, single-agent targeted cancer therapy is usually short-lived and thwarted by different resistance mechanisms (2). Here, we report the discovery of a JAK2/STAT5-evoked positive feedback loop that causes adaptive resistance to dual PI3K/mTOR inhibition. Mechanistically, PI3K/mTOR inhibition increased IRS1-dependent activation of JAK2/STAT5 and secretion of IL-8 in several cell lines and primary triple-negative breast tumors. Genetic or pharmacological inhibition of JAK2 abrogated this vicious feedback loop. Combined PI3K/mTOR and JAK2 inhibition synergistically reduced cancer cell viability *in vitro* as well as tumor growth *in vivo*, and decreased tumor seeding and metastasis due to its impact on the IL-8 receptor CXCR1+ tumor-initiating subpopulation of cells. We further found that combined PI3K/mTOR and JAK2 inhibition increased event-free as a well as overall survival of tumor bearing animals. This study reveals a new link between growth factor signaling, JAK/STAT activation, cytokine secretion and metastasis. Our results provide a rationale for combined targeting of the PI3K/mTOR and JAK2/STAT5 pathways in triple-negative breast cancer, a particularly aggressive and currently incurable disease.

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Y-Radiation promotes immunological recognition of cancer cells through Increased expression of cancer-testis antigens and promotes an intratumoral immune effector signature in human sarcoma

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Radiotherapy is an effective treatment for cancer. There is increasing evidence that radiotherapy not only induces DNA damage resulting in cell death, but also leads to alterations in the tumor microenvironment, particularly with respect to the immune infiltrate, thereby supporting tumor-specific immunity. We therefore investigated whether γ-radiation results in changes that are indicative for improved immune effector function. We compared the expression of CT-antigens and MHC-I in various cancer cell lines and fresh biopsies before and after *in vitro* irradiation (20 Gy). Furthermore, we retrospectively analyzed the expression of 35 immune response-related genes by qRT-PCR analysis and immunohistochemistry on paired formalin-fixed paraffin-embedded tumor sections in a cohort of 38 sarcoma patients before and after radiotherapy. *In vitro* irradiation of cancer cell lines and of fresh tumor biopsies induced a higher or *de novo* expression of different CT-antigens and a higher expression of MHC-I in a time- and dose-dependent fashion. Importantly, we showed that irradiation of cancer cells enhances their recognition by tumor-specific CD8+ T cells. Other forms of stress including hypoxia, hyperthermia and genotoxic stress did not have an impact on the expression of CT-antigens or MHC-I. Retrospective analysis of the expression of immune response-related genes in paired biopsies from sarcoma patients showed that radiotherapy resulted in an increase of many cell types and molecules that are characteristic for protective immunity, whereas those associated with immunoregulation were often downregulated. Furthermore, we found a relatively high expression of immune effectors and low expression of immune suppressors after radiotherapy in those patients who survived after radiotherapy in comparison to those who died, indicating that radiotherapy may essentially support the immune defense in sarcomas. Our findings suggest that radiotherapy promotes the immunological recognition of the tumor. Based on our data, we propose that a combination of radiotherapy with immunotherapy may improve the clinical outcome of standard therapies for sarcoma.

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Involvement of microRNAs in the DNA damage response: regulation of CtIP by the miR-17~92 cluster

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Incorrect repair of damaged DNA drives genomic instability, a hallmark of most human cancers. Therefore, an intact DNA damage response including accurate DNA repair represents a primary barrier to tumorigenesis. Recently, a crosstalk between the DNA damage response and the microRNA (miRNA) pathway became evident, indicating that DNA damage globally induces miRNA biogenesis and, vice versa, that numerous miRNAs modulate the expression of DNA damage response factors (1,2). The CtBP-interacting protein (CtIP) is crucial for the repair of DNA double-strand breaks by homologous recombination (3). Furthermore, CtIP acts as a transcriptional co-repressor and is involved in cell-cycle regulation. Balanced CtIP protein levels appear to be critical to prevent tumorigenesis, as Ctip heterozygous mice develop lymphomas, while its homozygous deletion results in embryonic lethality (4).
Since miRNAs regulate protein abundance by controlling mRNA stability

and protein translation, we aimed to identify miRNAs affecting the expression of CtIP. Our preliminary results identified CtIP as a novel target of the oncogenic miR-17~92 cluster. Furthermore, B-cell lymphomas, carrying an amplification of the 13q31-q32 genomic region harbouring the miR-17~92 cluster, displayed reduced CtIP protein and mRNA levels. In the future, we will investigate the mechanism of miRNA-mediated CtIP repression in more detail and its physiological significance for DNA repair. A comprehensive understanding of the regulatory circuit controlling CtIP protein abundance will provide further insights how its deregulation contributes to tumorigenesis.

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CtIP associates with FANCD2 to prime homology-directed repair of DNA interstrand crosslinks

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DNA interstrand crosslinks (ICLs) are the most deleterious lesions induced by chemotherapeutic drugs such as cisplatin and mitomycin C (MMC). Thus, multiple DNA repair activities are required for the resolution of ICLs, including the recognition by proteins of the Fanconi anemia pathway, nucleolytic incision, translesion DNA synthesis and, in a final step, homologous recombination (1). FANCD2 is a key player in the Fanconi anemia pathway as it orchestrates most of these processes. While there is quite a detailed understanding of how FANCD2 monoubiquitination coordinates ICL incision to generate DNA double-strand breaks, little is known about the potential contribution of FANCD2 to the repair of those double-strand breaks. On the other hand, CtIP is essential for the initiation of DSB resection, a general feature of HR during both mitosis and meiosis (2). To explore the functions of CtIP in ICL repair, we employed siRNA to downregulate the expression of CtIP in both FANCD2-proficient and -deficient cells. Cells lacking CtIP exhibit hypersensitivity to MMC and elevated levels of MMC-induced chromosomal aberrations. Furthermore, chromatin association of CtIP is dependent on FANCD2. Interestingly, we observed a physical interaction between CtIP and FANCD2. In summary, our data provide novel insights on the interplay of FANCD2 and CtIP in ICL repair and homologous recombination and their contribution to the maintenance of genome stability.

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T cell receptor affinity dependent induction of MicroRNA-155 drives efficient accumulation of CD8+ T cells by modulating cytokine signaling in response to virus and CD8+ cancer vaccination

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MiR-155 has multiple effects on immunity, but little is known about its role in CD8+ T cells (1). Testing different altered peptide ligands to activate naïve T cells, we found that miR-155 levels were dependent on the strength of TCR signaling. When we infected mice with lymphocytic choriomeningitis virus (LCMV) strains WE (acute) and CL13 (chronic infection), we observed that virus specific CD8+ T cell accumulation was strongly diminished in acute infection, whereas the long-term T cell response was abolished in the chronic infection, resulting in defective virus clearance and protection of immune pathology in miR-155-/- mice. Moreover, the tumor control upon transfer of tumor-specific naïve T cells and vaccination was inhibited in the absence of miR-155. Interestingly, miR-155 was intrinsically required for accumulation of effector cells by promoting proliferation and survival, providing competitive fitness to high affinity T cells. Importantly, we also detected a specific impairment of central memory T cell formation upon challenge with LCMV in the absence of miR-155. Previous work by others suggested suppressor of cytokine signaling-1 (SOCS-1) as a target of miR-155. Since effector CD8+ T cells are dependent on IL-2 for effective accumulation, we checked for impaired cytokine signaling, and found that pSTAT5 generation was affected in miR-155 deficient CD8+ T cells in response to IL-2, IL-7 and IL-15. Indeed, SOCS-1 was upregulated in miR-155-/- T cells. Our data suggest an important role for miR-155 for effector and memory CD8+ T cell responses and have implications for promoting potent cytotoxic T cell immunity for successful vaccination.

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Defining the mode of tumour growth by clonal analysis

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Recent studies suggest that cancer can be hierarchically organized as normal tissue containing cancer stem cells at the top of the cellular hierarchy. Cancer stem cells have been hypothesized to sustain tumour growth, to resist to chemo/radiotherapy and to be responsible for tumour relapse. Until now, cancer stem cells have been demonstrated in different human cancers by their ability to reform tumour upon transplantation which shows the potential of cancer cells in these experimental conditions but not necessarily reflect the actual fate of tumour cells in their native environment. Here, we made use of clonal analysis to unravel the mode of tumour growth *in vivo* in its natural environment (1). To this end, we used a genetic labelling strategy that allows individual tumour cells to express the YFP reporter gene and follow their fate over time. Interestingly, we found that in benign skin tumours, the majority of tumour cells have limited proliferative potential, while only a minority have the capacity to persist long term and divide rapidly, consistent with the marking of long lived cancer stem cells. Quantitative analysis, double-labelling experiments and detailed clonal fate data supports the existence of rapidly cycling cancer stem-like cells and a second population of more slowly cycling committed progenitors, mirroring the composition and hierarchy of the normal epidermis (2). By contrast, clonal dynamic in invasive squamous cell carcinoma is consistent with emergence of a single cancer stem cell population with limited potential for terminal differentiation. This study presents the first experimental evidence for the existence of cancer stem cells during unperturbed solid tumour growth.

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Targeted inhibition of the human papillomavirus 16 E6 oncoprotein

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Cervical cancer is the second most common cancer form in women and is caused by specific types of human papillomaviruses (HPVs), most commonly HPV16. The HPV E6 oncoprotein is regularly expressed in cervical cancer cells. E6 forms a complex with p53 blocking p53-mediated apoptosis. Targeted inhibition of E6 leads to p53 reconstitution and cell death of HPV-positive cancer cells. Thus, E6 is essential for the survival of HPV-positive cancer cells and the interference with E6 function should be an attractive therapeutic strategy (1). By screening of a randomized peptide expression library, a 15-mer peptide (pep11) was identified which specifically binds to HPV16 E6. It contains a novel E6-binding motif (2). Intracellular pep11 expression results in a strong inhibition of the colony formation capacity, specifically of HPV16-positive cells. This effect is linked to p53 reconstitution and induction of apoptosis. These findings show that the HPV16 E6 protein is amenable to intracellular inhibition by a small peptide, resulting in a therapeutically desirable phenotype. Thus, pep11 could provide an interesting model molecule for developing rational strategies to treat HPV16-positive (pre)neoplasias. Ongoing work focuses on (i) analyzing the pep11/E6 interaction at the biochemical level in order to define the E6 surface domain targeted by pep11, (ii) testing whether pep11 can enter HPV-positive cancer cells in a bioactive form following fusion to a protein transduction domain (PTD), and (iii) using the pep11/E6 interaction as a basis to screen for therapeutically useful small molecule inhibitors of E6.

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Endothelial CCR2 signaling induced by colon carcinoma cells enables extravasation via the JAK2-Stat5 and p38MAPK pathway

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Involvement of cytokines, chemokines and their receptors in the metastatic process is currently under extensive investigation. Increased expression of the chemokine CCL2 was shown to correlate with poor prognosis and increased tumor burden in metastatic breast, prostate and colon cancer. Recent results further suggest that tumor cell-derived CCL2 attracts Ly6Chi monocytes to facilitate tumor metastasis (1). However, the exact mechanisms through which CCL2 expression by tumor cells can enable the independent steps of metastasis upon the attraction of inflammatory monocytes remain elusive. Moreover, the role of the corresponding receptor CCR2 is only poorly characterized in metastasis.By using various experimental mouse models such as wild-type, knockout, cell type-specific transgenic and conditional knock-out mice, as well as by performing bone marrow reconstitution experiments, we could show an important role for endothelial CCR2 expression in tumor cell extravasation and metastasis. In a simplified approach focusing on the step of tumor cell extravasation using primary pulmonary endothelial cells, we could confirm our *in vivo* data and additionally focus on pathways downstream of CCR2. Finally, application of various inhibitors suggested that JAK2-Stat5 and p38MAPK are involved in the induction of lung vascular permeability, extravasation and therefore subsequent metastatic tumor growth. Our study identifies a novel molecular mechanism of chemokine-driven tumor cell extravasation involving the JAK2-Stat5 and p38MAPK pathway that goes beyond the so-far anticipated role of immune cell attraction. Our data therefore identify new targets for treating chemokine-dependent colon cancer metastasis.

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Gene expression analysis of a liver cancer mouse model reveals overlapping expression profiles with human hepatocellular carcinoma and identifies novel potential oncogenes

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Chronic liver diseases are characterized by a constant loss of hepatocytes. Mcl-1 is a crucial pro-survival factor for hepatocytes. Mice with hepatocyte-specific deletion of Mcl-1 (Mcl-1Δhep) reveal increased hepatocyte apoptosis, constant liver regeneration and spontaneous development of hepatocellular carcinoma. To understand apoptosis and proliferation-driven tumorigenesis, we analyzed the gene expression pattern in the Mcl-1Δhep model, and compared these to gene expression patterns in human liver tissues including hepatocellular carcinoma. RNA microarray of Mcl-1Δhep mice was performed and validated by RT-PCR, including several other genetic mouse models and human hepatocellular carcinoma of various etiologies. Tissue microarrays of human hepatocellular carcinoma were stained for candidate genes. RNA microarray uncovered significantly de-regulated genes in livers of 2 months old Mcl-1Δhep mice. Top 5 up-regulated genes were also found to be significantly up-regulated in Mcl-1Δhep mice derived hepatocellular carcinoma, these tumours in other genetic mouse models and in human hepatocellular carcinoma. Overexpression in the human hepatocellular carcinoma was confirmed on protein level by immunohistochemistry. The gene expression in Mcl-1Δhep mice and human tumor tissue show a high correlation and uncovered novel potential oncogenes of hepatocarcinogenesis. Our data suggest that human hepatocarcinogenesis follow a similar sequences of cell death, regeneration and carcinogenesis as in Mcl-1Δhep mice.

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High therapeutic efficacy and low toxicity using folate receptor targeted radionuclide tumor therapy

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Folate radioconjugates have previously been applied for imaging of folate receptor (FR)-positive cancer using SPECT and PET. However, a therapeutic application of folate radioconjugates was not envisaged due to significant renal uptake and hence a considerable risk of damage to the kidneys by particle-emitting radiation. Here we present a novel folate conjugate with albumin binding properties (cm09) allowing for the first time application of FR-targeted radionuclide tumor therapy [1]. *In vitro* evaluation revealed a clear inhibition of cell viability of FR-positive KB tumor cells in culture upon incubation with 177Lu-cm09. This effect was abolished if excess folic acid was co-incubated to block FRs. *In vivo* SPECT/CT imaging of mice using 177Lu-cm09 showed unprecedentedly high uptake of radioactivity in tumor xenografts and a reduction of the renal retention by >50% compared to previous radiofolates which lack an albumin binding entity. Studies were performed in mice whose tumor growth and body weights were monitored over two months upon therapy with 177Lu-cm09. Administration of 177Lu-cm09 (1 x 20 MBq/mouse) resulted in complete remission of tumors in most of the animals, and a significantly prolonged survival compared to untreated controls. Hence, modification of a folate conjugate with an albumin binding entity to enhance the blood circulation time resulted in a tremendous improvement of the tumor-to-kidney ratio enabling even successful radionuclide tumor therapy [1]. Excellent outcomes in terms of tumor regression mean a breakthrough in the field of therapeutic radioconjugates and hold promise for translation of this concept to the clinic.

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The genomic evolution of prostate cancer under the selective pressure of anti-androgen therapy

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The implementation of technologies such as array comparative genomic hybridization (aCGH) and next-generation sequencing has led to a deeper understanding of the genomic nature of cancer. However, these analyses have conventionally been done without respecting intra-tumor heterogeneity. Here, we applied a methodology that allows us analyzing the genomic profile of distinct tumor populations from individual tumors and their clonal evolution during the progression to castration-resistant disease(1). Matched pre- and post- hormone treated fresh frozen and/or formalin fixed prostate cancer samples were selected. Tumor populations were flow-sorted according to their DNA content. Sorted tumor populations were subjected to whole genome aCGH and to full exome sequencing. The analysis of matched tumor specimens allowed us to identify two particular patterns of tumor evolution during the progression after treatment: First, a more parallel pattern of tumor evolution, in which the ancestor population is breeding multiple aneuploid tumor clones. In this case, only the 2N ancestor population is able to withstand therapy by the acquisition of few specific genomic aberrations whereas the aneuploid populations are eradicated. Second, a more sequential pattern of tumor evolution with a tumor population that evolves out of a continuous line of clones. This population shows increasing signs of genomic instability over time, with a punctual event of chromothripsis (a massive destruction and rearrangement of chromosomal structures) resulting in castration-resistant tumor populations. We conclude that genomic profiling of distinct clonal tumor populations during prostate cancer progression allows for the analysis of intra-tumoral heterogeneity and the underlying evolution towards castration-resistance.

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Expression profiling analysis of Src-transformed cells reveals novel metastasis-associated genes

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The sarcoma protein c-Src is the oldest known proto-oncogene. Despite intensive investigation of c-Src and the viral oncogene protein v-Src, many details about v-/c-Src-induced oncogenic transformation remain unknown. Overexpression and/or activation of c-Src are hallmarks of many human tumors and associated with increased metastatic activity [1]. We have established a cellular model for c-Src metastasis by stably expressing a constitutively active c-Src mutant in human cell lines. The mutated c-Src, with a C-terminal single point mutation, is disrupted from binding to the PDZ domain of a tumor suppressor protein [2]. The metastatic cells have been subjected to RNA profiling by Illumina deep sequencing, which allowed us to obtain the transcriptional landscape of c-Src mutant-regulated genes in unprecedented detail on a genome-wide scale. mRNA levels of c-Src mutant-expressing cells were compared with c-Src wild-type-expressing and control cells, allowing us to define a set of differentially expressed genes which were functionally annotated by Gene Ontology enrichment analysis and assigned to signaling pathways. More than 400 genes were identified, involved in a broad range of biological functions including apoptosis, cytoskeleton remodeling, adhesion and migration and protease activity, confirming the role of the Src mutant as a master regulator of gene expression and its involvement in metastasis. Literature mining revealed that ~20% of differentially-expressed genes identified in this study have previously not been associated with cancer or metastasis. This set of genes provides previously unknown potential markers for metastasis as well as novel putative targets for diagnostics or cancer therapy.

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Establishment of serum autoantibody signature for early gastric cancer detection

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Although the role of B cell anti-tumour immune response is not completely understood and remains controversial, autoantibodies specific to tumour-associated antigens (TAAs) at varying frequencies are found in nearly all cancer patients from early to late stages, and multiplexing of autoantibody reactivity have recently been shown as relevant for cancer diagnosis. In our previous study we identified 1350 different TAAs by applying T7 phage display SEREX approach to melanoma, prostate and gastric cancer (GC). Among them there were some new and well-known TAAs and autoantigens, and a high proportion of unnatural peptides. All the recombinant phage clones were used to generate phage-displayed antigen microarrays that were probed with sera from 240 cancer patients and 100 healthy donors to obtain the serum-reactivity pattern for each antigen. Methods for data normalisation and cut-off determination were elaborated enabling to determine seropositive signals and rank antigens by the signal intensity and frequency of reactivity. In the current study 86 top-ranked GC reactive antigens were selected for the generation of focused array that was tested with sera from 235 GC, 154 peptic ulcer and gastritis patients and 213 controls. A 'serum score' was calculated for each serum by summing up signal intensities for 45 top-ranked antigens, and it could discriminate GC from healthy individuals with AUC of 0.79 (sensitivity 59% and specificity 90%), GC and peptic ulcer with AUC of 0.76, and GC and gastritis with AUC of 0.64. Moreover, it could detect early and advanced GC with equal sensitivity, and was associated with shorter overall survival (HR 4.48, P=0.00045).

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High-throughput mutation profiling of cutaneous T cell lymphma reveals RAS mutations sensitizing tumors toward inhibition of the RAS pathway

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Cutaneous T-cell lymphomas (CTCLs) are malignancies of skin-homing T lymphocytes, which thusfar have not been investigated for oncogenic mutations. We screened 90 biopsy specimens from CTCL patients (41 mycosis fungoides, 36 Sézary syndrome, and 13 non-mycosis fungoides/Sézary syndrome CTCL) for somatic mutations using OncoMap technology. We detected oncogenic mutations for the RAS pathway in 4 of 90 samples. Two patients harbored a KRAS(G13D) mutation and two a NRAS(Q61K) amino acid change. All mutations were found in stage IV patients (4 of 42) who showed significantly decreased overall survival compared with stage IV patients without mutations (P = .04). In addition, we found a NRAS(Q61K) mutation in the CTCL cell line Hut78. Knockdown of NRAS by siRNA induced apoptosis in mutant Hut78 cells, but not in CTCL cell lines lacking RAS mutations. The NRAS(Q61K) mutation sensitized Hut78 cells toward growth inhibition by the MEK inhibitors U0126, AZD6244, and PD0325901. Furthermore, we found that MEK inhibitors exclusively induce apoptosis in Hut78 cells. Taken together, these finding indicate that RAS mutations do occur at late stage of CTCL patients and that our preclinical results suggest that such late-stage patients may profit from MEK inhibitors.

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MicroRNA-mediated down-regulation of NKG2D ligand expression reduces glioma cell immunogenicity

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Malignant gliomas are intrinsic tumors of the brain with a dismal prognosis despite multi-modal therapy. They are characterized by diffuse infiltration of the surrounding healthy brain tissue, well-adapted to hypoxic conditions and regarded as paradigmatic for tumor-associated immunosuppression. NKG2D is the most important activating receptor of natural killer cells. It recognizes at least 6 ligands (NKG2D-L) which are induced after malignant transformation and cellular stress. Recent findings demonstrate that endogenous small RNA molecules known as microRNAs are also involved in the control of NKG2D-L expression leading to reduced NK cell recognition. In the present work we aimed at evaluating the impact of miRNA on the expression of NKG2D-L in glioma cells including stem-like glioma cells. Out of 6 miRNA which may target NKG2D-L in glioma cells, 3 candidates are expressed in various glioma cell lines. Locked nucleic acid inhibitors were used to silence the expression of single miRNAs. miRNA silencing results in an up-regulation of NKG2D-L on the surface of glioma cells. This increase in NKG2D-L expression translates into increased susceptibility to NK cell-mediated lysis in a killing assay. Targeting miRNA may therefore be a novel approach to increase the immunogenicity these lethal tumors.

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Influence of ALDH activity in the stem cell properties of neuroblastoma cells

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Neuroblastoma (NB) is the most aggressive extracranial childhood tumour. The existence of a cell hierarchy with a minor sub-population of so-called cancer stem cells (CSCs) may be at the origin of the typical heterogeneity of this tumour. However this CSC population has not yet been formally identified in NB. A microarray time course analysis of serial NB spheres passages allowed us to specifically \"profile\" the NB stem cell-like phenotype and identify, among others, ALDH1A2 as a potential tumour-initiating cells (TIC) marker for NB. Three ALDH1 isoforms involved in development and in the synthesis of retinoic acid have been already identified as functional stem cell markers in leukaemia and breast cancers. By using the Aldefluor flow cytometry-based assay, we confirmed that increased ALDH activity was observed in a restricted number of cells upon NB sphere passages, suggesting that a pre-existing ALDHhigh cell subpopulation was selected during the self-renewal process. Conversely, specific inhibition of ALDH activity resulted in significant reduction in the self-renewal capacity and in the clonogenic potential of NB cells. Moreover, NB cells resistance to various drugs was partially abolished by inhibition of ALDH activity, however in a cell line- and drug-dependent fashion. ALDH activity-based cell sorting is now used to investigate the functional behaviour of ALDHhigh and ALDHlow cell subpopulations. ALDHhigh active cells express higher amount of MDR1 and/or CD133, two NB-TICs profile markers. Whether ALDH activity, combined with TICs-profile markers, identifies a tumour-initiating cell population involved in the maintenance of the tumour is currently explored.

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Hedgehog signaling controls Gas5 long noncoding RNA in malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) is an aggressive cancer that is primarily caused by inhalation of asbestos fibers and is a relatively chemoresistant malignancy. The median survival duration of MPM is <1 year and the 5 year survival rate <1%. Long noncoding RNAs (lncRNA) are a class of RNAs >200 nucleotides in length, that do not code for protein, but make up >90% of the human genome. Earlier experimental studies showed Gas5 (growth arrest specific transcript 5) deletion in asbestos driven mesothelioma. The function of Gas5 is not well known, but it seems to act as glucocorticoid receptor decoy. Our aim is to investigate the possible role of the Gas5 lncRNA in the growth of MPM. Primary MPM cultures grown in serum-free condition in 3% oxygen were used. Doxycycline inducible shGas5 clones were generated from ZL55SPT cells. Gene expression in tumors, non-tumoral samples and primary culture was quantified by qPCR. Our results reveal that a six fold significant higher expre ssion level of Gas5 was observed in the tumor compared to non-tumoral tissue. However, upregulation of Gas5 was observed when primary MPM cells were growth arrested by inhibiting hedgehog (Hh) signaling while overexpressing downstream Hh target Gli1 decreased Gas5 expression. Gas5 silencing induced by doxycycline addition led to increased expression of the glucocorticoid-inducible genes GILZ and SGK-1. In conclusion, Gas5 lncRNA is significantly upregulated in MPM tumors. Its expression is maintained in MPM primary cultures and changes according to Hh regulated-cell proliferation status. Silencing of Gas5 modulates the expression of glucocorticoid responsive genes. Further studies will investigate whether these changes are associated with the modification of cell growth and whether Gas5 expression has a prognostic value in MPM.

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Calretinin is essential to mesothelioma cell lines and may be a potential new target for gene therapy

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Malignant mesothelioma (MM) are highly aggressive asbestos-related neoplasms, which show strong chemotherapy-resistance and there is no effective cure for MM so far. Calretinin (CR) is widely used as a diagnostic marker for epithelioid and mixed (biphasic) type mesothelioma [1], but still little is known about the putative function(s) of CR in tumorigenesis. CR partially protects against asbestos-induced acute cytotoxicity mediated by the AKT/PI3K pathway and furthermore, SV40 early region genes (large and small T antigen) are able to up-regulate CR in mesothelial cells [2]. However, the precise role of CR in mesothelioma is still unknown. Here we show that down-regulation of CR via lentiviral-mediated shRNA significantly decreased the viability and proliferation of mesothelioma cells *in vitro*. The effect was stronger in epithelioid cells including cell lines MSTO-211H and ZL55. A weaker and also delayed effect was observed in mesothelioma cells of the mixed (SPC111 and SPC212) a s well as the sarcomatoid type (ZL34). The specificity of the effect was confirmed by stable eGFP-CR expression in mesothelioma cell lines and subsequent down-regulation. Depletion of CR led these cancer cell lines to enter apoptosis within 72 h post-infection via strong activation of caspase 3/7. Our results demonstrate that down-regulation of CR had a strong effect on the viability and that CR is "essential" for cells derived from epithelioid and biphasic mesothelioma. We anticipate these findings to reveal calretinin as a highly interesting new therapeutic target for gene therapy in mesothelioma of especially the epithelioid, but also of the biphasic and sarcomatoid type.

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Aging-associated intrinsic and extrinsic factors control hematopoietic stem cell behavior

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Life-long self-renewing hematopoietic stem cells (HSCs) repetitively contribute to replenishment of mature blood cells. With aging HSCs show reduced self-renewal, less efficient bone marrow –homing capacity, and myeloid-skewed differentiation. We have demonstrated that HSCs with increased proliferative history tend to be dormant in a permissive environment1, suggesting that a cell-intrinsic drive towards dormancy is imprinted on HSCs by increasing divisional history in aging. Here, we tackle the questions what extrinsic and intrinsic factors determine HSC behaviour at cellular and molecular level. We performed *in vivo* HSC divisional tracking where CFSE-labeled young or aged HSC-containing fractions (LKS) were transferred into steady-state young or aged recipients. Eight week BM analysis showed that young LKS proliferated faster than old LKS independent on BM environment, while both young and aged LKS appear to be more dormant in old environment. To test function of HSC with distinct divisional history, dormant (0-divided at 8 weeks) or cycling LKS (>5-divided) were isolated and transplanted into lethally irradiated mice. Dormant aged HSCs irrespective of environment favour myelopoiesis. In contrast, cycling aged HSCs that had been exposed to young environment showed balanced lineage repopulation as do both cycling and dormant young HSCs. These findings demonstrate that extensive proliferative history imprints a cell dormancy program on HSCs that is associated with myeloid-biased differentiation, and that the differentiation program can be modulated via environmental cues. Understanding of how cell cycle and fate is determined and altered upon aging will open a new avenue for regenerative medicine.

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Drug response profiling to identify new targets in refractory leukemia

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Functional correlations of oncogenic lesions with drug response profiles are ill defined for childhood acute lymphoblastic leukemia (ALL)[1]. We have developed an automated microscopy-based image analysis approach based on co-cultured ALL cells on human bone marrow mesenchymal stroma cells. Using this platform, we provide evidence for activity profiles of a selection of bioactive molecules and new therapeutic agents in samples from distinct ALL subgroups. We compiled a 110 compound library containing chemotherapeutic agents as well as compounds in preclinical and early clinical development. We obtained patient specific response patterns by comparing samples from distinct subtypes of precursor B-cell ALL including samples with translocation t(17;19) - a highly resistant subtype and t(1;19)- a subtype that is associated with good outcome, as well as T-ALL samples. This approach identifies classes of compounds with a high degree of activity across multiple samples, such as inhibitors of mTOR or chromatin remodelling. Activity of a new Notch1 inhibitor correlated with activated Notch1 signalling, further illustrating the potential of this platform. Our data indicate presence of specific drug response profiles in genetically distinct ALL subtypes and suggest that our platform will be useful for *in vitro* ALL drug profiling. We expect to derive relevant functional information directly from individual patient samples, which may mirror perturbations in relevant cellular programs. We will evaluate this system for the identification of novel anti-leukemic compounds, for the definition of predictive profiles of clinical response and will use combinatorial drug profiling to identify new alternatives for the treatment of refractory ALL.

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Comparative proteomics identifies a prognostic marker for relapse in intermediate risk acute lymphoblastic leukemia

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A better description of the leukemia cell surface proteome will provide new opportunities for biomarker discovery. A semiquantitative [1] comparison of the cell surface proteome of samples with *de novo* resistant acute lymphoblastic leukemia (ALL) and with chemosensitive disease identified members of the Vanin (VNN) family of proteins, in particular VNN2, to be preferentially present in resistant samples. Protein detection correlated with mRNA expression, which enabled us to evaluate VNN2 in a large retrospective cohort of diagnostic ALL samples. Strikingly, very high levels of VNN2 were detected only in samples with intermediate or high risk ALL, identified by persistence of minimal residual disease (MRD) during chemotherapy. In the IR risk group, high VNN2 expression was independent of other clinical or genetic risk factors and represents the strongest risk factor identified for this cohort. Persistence of high VNN2 in matched xenograft material shows that high expression of VNN2 is a stable transplantable feature, suggesting that VNN2 correlates with an underlying leukemia specific aberration. The gene expression signature associated with VNN2 in xenograft samples was detected with significant enrichment in expression profiles from independent patient cohorts. Strikingly, VNN2 expression appears to be higher at relapse in paired presentation and relapse samples supporting its association with resistant leukemia. VNN2 was shown to contribute to lymphocyte trafficking and to inflammatory responses, while its role in leukemia cells remains unknown. Collectively our data identify VNN2 as novel risk factor and strongly suggest that this marker identifies a specific ALL entity with a distinct deregulation of cellular programs.

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Detection of circulating metastasis-initiating breast cancer cells by EPCAM, CD44, CD47 and MET

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It has been hypothesized that, in the blood of carcinoma patients, a subpopulation of circulating tumor cells (CTCs) must act as functional metastasis-initiating cells (MICs) (1). However, although the presence of CTCs often correlates with poor prognosis (2-3), the existence of MICs within CTCs has not been demonstrated, nor has their phenotype been characterized. Here we demonstrate that luminal breast cancer CTCs do contain MICs which initiate bone and liver metastases in mice. These MIC-containing EPCAM+-CTCs express CD44, CD47 and MET. The frequency of CD44+MET+CD47+EPCAM+ patient CTCs increased parallel to disease progression and correlated with decreased overall-survival. In addition, tissue-microarray analysis revealed co-expression of CD47 and MET as a novel independent predictor of poor overall-survival, which strongly correlates with metastasis. These data uncover the first markers for functional blood circulating MICs, opening new avenues for the design of diagnostic tools and development of rational-based approaches to target metastasis in breast cancer.

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Dissecting the specific cellular immune response towards MAGE-C1/CT7 in myeloma patients

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Multiple myeloma cells are susceptible to cellular immunity; therefore finding immunogenic and selectively expressed antigens for immunotherapy is warranted. Between the known tumor antigens, MAGE-C1 (CT7) is the most frequently expressed cancer-testis (CT) antigen in multiple myeloma lesions being found in more than 50% of cases (1) and represents an ideal target for immunotherapy for its exclusive expression on cancer cells and immunogenicity (2). In order to investigate the spontaneous and specific cellular immune response towards CT7, we analysed the PBMC from 18 multiple myeloma patients whose bone marrow lesions expressed CT7. We detected CT7-specific memory CD4+ T cells in 3 out of 18 MM patients. In 2 out of 3 patients this response could be detected only in the absence of Tregs, thus demonstrating that Tregs control CD4+ CT7-specific T cell responses in multiple myeloma. We were able to further characterise the CT7-specific CD4+ T cell clones and demonstrate that they recognize naturally processed CT7 and defined the minimal epitopes. These CT7-specific CD4 T cells moreover provide help for humoral immune response since all three patients with a T cell response had CT7-specific IgG antibodies, demonstrating an integrated immune response towards CT7 in myeloma patients. These results are of clinical relevance since they show, for the first time, that multiple myeloma patients develop T cell immune responses towards CT7 and that this response might be suppressed *in vivo* by naturally occurring immune suppression. Therefore, targeting CT7 with simultaneous use of immune suppression antagonists is of great promise for multiple myeloma immunotherapy.

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MicroRNAs as potential targets and anticancer agents

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Tumor growth is associated with regular shifts in microRNA (miRNA) expression pattern. Cancer cells down-regulate expression of miRNAs miR-15a/16, miR-17-5p, miR-31, miR-125a/b, miR-143 and miR-145 that silence proliferative and anti-apoptotic genes [1]. miRNA miR-17-5p can target transcripts of genes coding proliferative signal pathway components E2F1/2, STAT3, Rb, p107, p130, ErbB3. miR-15a and miR-16 can suppress genes encoding transcription factor E2F3/7, cyclin-dependent kinase CDK6 and main anti-apoptotic gene bcl-2. E2F2, STAT3, erbB2 and bcl-2 gene transcripts carry miR-125a/b binding sites. miR-143 can suppress abl2, erbB3 and bcl-2 genes. miR-145 targets transcripts of RASA1, RASA2, erbB3 genes. miR-320 can suppress E2F1/3, RASA1 and CDK6 genes. Up-regulated miRNAs miR-21, miR-23a/b, miR-155, miR-206, miR-221/222 suppress genes encoding cell cycle inhibitors as well as key elements of cell differentiation network [1]. miR-21 silences gene encoding nuclear factor NF-IB that inhibits NF-κB. miR-155 can suppress genes coding transcription factors EBF1, CEBPB, Ets1, Meis1 and PU1. miR-23a/b targets transcript of genes encoding NFIB, MITF, Blimp1 and receptor FAS. miR-29a/b can suppress genes coding transcription factor T-bet (TBX-21), proapoptotic agent Bak1 and receptor TNF-R1. miR-221 and miR-222 silence genes encoding cell cycle inhibitors p27 and p57 as well as receptor c-Kit, transcription factors Ets1 and Fos. Shifts in miRNA expression pattern can themselves cause reactivation of cell oncogenes and antiapoptotic genes as well as repression of cell cycle inhibitor genes [1,2]. Therefore, treatment with miRNAs, which are down-regulated in cancer cells, can restore normal activity of proliferative as well as apoptotic pathways. Up-regulated miRNAs may be targets for antisense or regulatory treatment.

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Blockade of CD39- and CD73-dependent ATP-depletion in ovarian carcinoma - a possible immunological adjuvant for conventional therapies

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Cellular expression of CD39 and CD73 contributes to immunosupression as these ectonucleotidases convert immune-stimulatory extracellular ATP into immunosuppressive adenosine. This was primarily described as effector mechanism for regulatory T cells, but may be more important in the tumor microenvironment as we recently could confirm for ovarian cancer (OvCA). Investigating the regulation of the ectonucleotidases\' expression we now have observed that approaches clinically used to treat OvCA (namely, application of doxorubicine or irradiation) *in vitro* influence CD73 levels of OvCA and immune cells. In this study we show how this treatment-induced change in the ATP/adenosine ratio modulates the effector function of different immune cells. Furthermore, we investigate the potential benefit of clinically available small molecule inhibitors for CD39 and CD73 that could relieve immunosuppression in the tumor microenvironment especially in combination with common treatment regimes.

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In vitro and *in vivo* characterization of mouse glioma cancer stem cells

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Glioblastomas are highly aggressive primary brain tumors in adults and despite multimodal treatment of surgery followed by radiotherapy and chemotherapy, commonly temozolomide (TMZ), the median survival is only 14.6 months. Conventional therapy is thought to be ineffective against a subpopulation of cells, termed glioma cancer stem-like cells that were found to have an extensive role in tumor progression. Cells derived from high but not low-grade gliomas share several stem-cell properties with the neural stem cells (NSC) including: selective expansion in NSC culture conditions that promote sphere formation; expression of radial glia NSC markers including nestin, GFAP, Musashi-1; and multipotency. In addition they have the ability to give rise to orthotopic tumors that recapitulates immunological, histological and morphological features of the initial human tumor when injected into mice. However, there have been growing arguments against the use of sphere cultures as a means to enrich for a more tumorigenic phenotype. In our study we use both *in vitro* and *in vivo* approaches to address the question of whether the glioma CSC (SC) derived from sphere cultures are more tumorigenic than the glioma non-stem (NS) cells in four different mouse glioma cell lines (SMA-497, SMA-540, SMA-560 and GL261). We also test the sensitivities of these two cell types to increasing doses of ionizing irradiation and TMZ. Our *in vitro* results indicate that NS share several stem-cell properties with SC. However, sphere culture conditions seem to have a protective role against increasing irradiation and TMZ concentrations. Interestingly, NS had higher *in vivo* tumorigenicity than SC in all but the SMA-560 glioma cell line, indicating sphere cultures are not always synonymous with increased tumorigenicity.

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Breast cancer cells acquire stem cell-like properties upon contact with functionally impaired T cells

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Breast cancer is the most common malignancy in women and patients are prone to relapse. This has been ascribed to a small subpopulation of so-called tumor initiating or cancer stem cells (CSC) characterized by increased clonogenicity, sphere formation and tumor initiation capacity. We investigated interactions between T cells and breast cancer stem cells which can be recognized by the marker combination CD44highCD24low. Upon cell-cell-contact with polyclonally expanded and activated CD4+ and CD8+ T cells from healthy donors, the CSC population in MCF7 breast cancer cells is not only spared from killing and thus enriched by selection, it is further induced. This CSC-inducing effect was considerably enhanced when the degranulation of T cells was inhibited by pretreatment with concanamycin A. Interestingly, serum from patients with metastasized breast cancer also promoted a non-lytic phenotype in immune cells from healthy donors. After co-culture, MCF-7 cells displayed increased clonogenicity and spheroid formation *in vitro*. Changes in gene expression suggested that this de-differentiation might occur via epithelial-to-mesenchymal transition. In line with this hypothesis, the effect could be attenuated by inhibition of TGF-beta, TNF-alpha-, hedgehog- and NF-κB signaling. We further observed stem-cell-inducing properties in T cells from breast cancer patients, suggesting that induction of a stem-cell-like phenotype is an alternative and undesirable outcome of the interaction between immune cells and tumor cells. However, modulation of this effect seems possible since several of the involved pathways represent potentially druggable targets for therapy. Further studies will now have to evaluate the distinct molecular interactions mediating this cell-contact-dependent CSC induction.

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Loss of the tumor suppressor VHL leads to aneuploidy mediated by microRNA 28 and Mad2 driving kidney cancer development

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The von Hippel-Lindau (VHL) tumor suppressor is inactivated in the large majority of clear cell renal cell carcinomas (ccRCC). However its tumor suppressive function is incompletely understood. The VHL protein has multiple functions with relevance to ccRCC development, including targeting the hypoxia inducible factor HIF for ubiquitin-dependent degradation and stabilizing microtubules. Recently, we linked VHL loss to the development of aneuploidy *in vitro* via reduced expression of the mitotic checkpoint protein Mad2. Using ribosome profiling, we present in this study that VHL is affecting Mad2 translation. We show that this is mediated via the 3' untranslated region (3'UTR) of Mad2 and further identified the microRNA miR-28-5p as a potential regulator. We could show that miR-28-5p, when overexpressed, leads to chromosome segregation errors, and as a consequence, to aneuploidy. Furthermore, a mutated mir-28-5p binding site renders the Mad2' 3'UTR independent of VHL control, suggesting a mechanistic link. In line with this, we demonstrate that miR-28 is transcriptionally upregulated upon VHL loss in primary and cancer cell lines, as well as in a kidney specific VHL knockout mouse model, and in samples from ccRCC patients. Using a bioinformatics approach, we could establish that ccRCCs develop aneuploidy early in their development and that the extent of aneuploidy correlates with miR-28-5p expression. Finally, we present that combined loss of VHL and Mad2 heterozygosity in the kidney leads to the development of precancerous lesions. Together, these results suggest a novel mechanism of aneuploidy generation contributing to carcinogenesis in VHL-negative tumors.

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Unraveling the mechanisms towards IL-12 mediated glioma rejection

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Glioblastoma (GB) is the most common and malignant of the glial tumors, having extremely low cure rates. Median survival of patients diagnosed with GB ranges between 12-15 months, despite aggressive surgery, radiation and chemotherapy (1). One treatment approach is to reverse the suppressive tumor microenvironment into a pro-inflammatory microenvironment with increased antigen-presentation and subsequent rejection. Using a mouse model of experimental glioma, our lab has employed interleukin-12 (IL-12) to elicit an antitumor immune response. Intratumoral IL-12 application has been investigated in different experimental brain tumor immunotherapy models, leading to long-term antitumor immunity (2). Moreover, it induces a strong infiltration of activated CD4+ and CD8+ T cells (3). However, while the ultimate effectors are well established, the contribution of IL-12 in priming this adaptive immune response remains elusive. This research is designed to dissect the mechanisms leading to IL-12 mediated glioma rejection, investigating the relationship between the brain-resident and systemic parts of the immune system. The research anticipated will include the identification of the responsible antigen presenting cell (APC) subset as well as elucidation of the site of antigen presentation to T cells. Further investigation will involve immunosuppressive mechanisms which contribute to the abrogation of effector mechanisms. Understanding the underlying priming mechanisms of IL-12 mediated glioma rejection will be critical for the development of immunotherapies against glioma.

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Molecular mechanism of innate lymphocyte cells on cytokine-induced tumor suppression

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Malignant melanoma is a particularly aggressive tumor due to its metastasizing propensity and is the cause of most skin cancer related deaths worldwide. Interleukin-12 (IL-12) has consistently been demonstrated to possess potent tumor suppressing activity in murine melanoma models, however systemic administration to melanoma patients led to severe adverse effects. Our laboratory has also described that local IL-12 delivery results in strong tumor suppressive activity mediated by innate lymphoid cells [1]. Although the molecular underpinnings behind this observation remain elusive, we hypothesize that IL-12 initiates tumor suppression through innate lymphoid cells by changing the tumor microenvironment to be more conducive to immune invasion. The aim of this research is to specifically (1) systematically unravel the impact of local IL-12 treatment and combinatorial therapies by blocking immunosuppressive molecules in prophylactic and therapeutic settings in distinct murine melanoma models and (2) to determine the molecular mechanism underlying the IL-12 mediated tumor suppression by identifying and characterizing molecular targets of IL-12 signaling on innate lymphoid cells. A better understanding of the role of IL-12 and innate lymphoid cell function in tumor suppression may lead to new immunotherapeutic intervention opportunities by manipulating the potential of IL-12 signaling to modify the tumor microenvironment.

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Links between Senataxin, defective in AOA-2, and common fragile sIte instability

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Defects in DNA repair have been linked to genomic instability, cancer predisposition, premature aging and neurological disorders. One major interest of our lab is to understand the molecular defects that lead to the neurodegenerative diseases collectively termed as ataxia with oculomotor apraxia (AOA). Three classes of AOA have been identified so far, namely AOA-1, AOA-2 and AOA-3. The molecular defect in AOA-2 maps to the SETX gene at chromosome 9q34. SETX encodes a 2677 amino acid protein, Senataxin, which has a predicted seven-motif helicase domain at its C-terminus, typical of the superfamily 1 of DNA/RNA helicases. Recent evidence suggests that Senataxin acts to resolve R-loops that arise during pause-dependent transcription termination. The goal of this work is to further understand the molecular functions of Senataxin in the maintenance of genomic stability. Using cell viability assays, we found that cells depleted for Senataxin were sensitive to treatment with agents that cause either replication stress or induce the formation of RNA/DNA hybrids. In mammalian cells, Senataxin localises to G1-nuclear bodies where it colocalises with 53BP1, a key marker in the DNA damage response. We find that these Senataxin/53BP1 nuclear bodies increase in number after mild replication stress. In addition, depletion of Senataxin elevates the occurrence of 53BP1 nuclear bodies and the frequency of DNA breaks. Furthermore, in Senataxin deficient cells, the DNA damage markers 53BP1 and γ-H2AX were found to be enriched at many common fragile sites. These data lead us to suggest that Senataxin plays an important role in the maintenance of common fragile site stability, possibly by the resolution of R-loop structures that are formed at those sites by the collision of transcription and replication machineries.

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Friedreich's ataxia-associated GAA repeats induce replication fork reversal and unusual molecular junctions in human cells

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Expansion of GAA/TTC repeats is the causative event in Friedreich's ataxia. GAA repeats were shown to hinder replication in model systems, but the mechanisms of replication interference and expansion in human cells remained elusive. To study *in vivo* replication structures at GAA repeats, we designed a novel plasmid-based system which permits analysis of human replication intermediates by 2D-gel electrophoresis and electron microscopy. We found that replication forks transiently pause and reverse at long GAA/TTC tracts in both orientations. Furthermore, we identified replication-associated intramolecular junctions between GAA/TTC repeats and other homopurine-homopyrimidine tracts associated with breakage of the plasmid fork not traversing the repeats. Finally, we detected postreplicative, sister-chromatid hemicatenanes that were converted into persistent homology-driven junctions at GAA/TTC repeats. These data establish that GAA/TTC tracts interfere with replication in humans and strongly implicate postreplicative mechanisms in trinucleotide repeat expansion.

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Loss of cutaneous TSLP-dependent immune responses skews the balance of inflammation from tumor protective to tumor promoting

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Inflammation can promote or inhibit cancer progression. In this study we have addressed the role of the proinflammatory cytokine thymic stromal lymphopoietin (TSLP) during skin carcinogenesis. Using conditional loss- and gain-of-function mouse models for Notch and Wnt signaling, respectively, we demonstrate that TSLP-mediated inflammation protects against cutaneous carcinogenesis by acting directly on CD4 and CD8 T cells. Genetic ablation of TSLP receptor (TSLPR) perturbs T-cell-mediated protection and results in the accumulation of CD11b(+)Gr1(+) myeloid cells. These promote tumor growth by secreting Wnt ligands and augmenting β-catenin signaling in the neighboring epithelium. Epithelial specific ablation of β-catenin prevents both carcinogenesis and the accumulation of CD11b(+)Gr1(+) myeloid cells, suggesting tumor cells initiate a feed-forward loop that induces protumorigenic inflammation.

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Mechanistic control of replication fork reversal and restart upon chemotherapeutic treatments

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We have recently identified fork reversal – that is, the conversion of replication forks into four way junctions - as a global, evolutionary conserved response to replication stress induced by topoisomeraseI inhibitors (1). This cellular response, which protects cells from drug-induced chromosomal breakage, requires poly(ADP-ribosyl)ation (PAR) mediated by specific PAR polymerases, in order to prevent untimely fork restart by specific cellular activities (1,2). We have recently investigated the relevance of this regulation during unperturbed S phase, studying the effects of PAR accumulation by depletion of the only PAR degrading enzyme identified to date, namely PAR glycohydrolase (PARG). We show that PARG depletion affects cell proliferation and nucleotide incorporation, resulting in the accumulation of various markers of DNA damage and repair (γH2AX, 53BP1, Rad51 and RPA foci), even in the absence of exogenous genotoxic stress. Single molecule analysis reveals replication fork slow down, fork reversal and single stranded DNA accumulation in PARG-depleted cells, at similar levels as upon mild chemotherapeutic treatment. This suggests that PAR degradation is required to drive restart of replication forks transiently reversed by endogenous obstacles and to avoid unscheduled recruitment of DNA repair factors at unusual replication intermediates. We are in the process of investigating whether fork reversal is a common response to various types of chemotherapeutic treatments and to test the mechanistic contribution of several candidate cellular factors in the process of fork reversal and restart.

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The ETS factor ESE3/EHF controls differentiation and stemness in human prostate tumors

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Cancer stem cells (CSCs) are considered major elements in initiation, progression, disease recurrence and treatment failure in many human cancers. Here we show that ESE3/EHF, an ETS family transcription factor normally expressed in prostate epithelial cells, controls cell differentiation and stem-like potential. ESE3/EHF knockdown in immortalized prostate epithelial cells induced epithelial-to-mesenchymal transition (EMT), stem-like features, tumour-initiating and metastatic properties. Conversely, re-expression of ESE3/EHF in prostate cancer cells reduced cell transformation, stem-like properties and tumorigenic potential. Gene expression profiling studies showed that ESE3/EHF acted as key node controlling expression of a large network of genes and that its loss led to a complex reprogramming of the cell transcriptome. Mechanistically, ESE3/EHF maintained in a repressive state key EMT and CSC genes in normal epithelial cells, which were derepressed in cancer cells with ESE3 loss. Analysis of clinical tissue samples by IHC and multiple prostate cancer microarray datasets showed that loss of ESE3/EHF expression defines a tumor subgroup ($ESE3^{low}$ tumors) with distinctive aggressive features. ESE3 tumours were enriched of EMT and CSC genes compared to the bulk of prostate tumors and could cluster together. Consistent with these biological features, we found that tumors with low ESE3/EHF expression had higher recurrence and reduced overall survival compared to ESE3 expressing tumors in surgically treated patients. Collectively, these data point to a key role of ESE3 in controlling prostate cancer stem cells and identify a group of tumors with aggressive features that might benefit of alternative therapeutic strategies.

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. Frederique 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 the cancer research. Through her personal committment, cancer research in Zurich has been significantly strengthened. President Jacques Chirac of France elected 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 at the 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 absolute 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.

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The Foundation Board administers the Foundation and determines policy. The Foundation Board makes final decissions regarding Prizes and Symposia.

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Wissenschaftlicher Beirat

Der Wissenschaftliche Beirat nominiert Kandidaten für den Charles Rodolphe Brupbacher Preis für Krebsforschung und empfiehlt Redner für die wissenschaftlichen Symposien.

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The Scientific Advisory Board nominates candidates for the Charles Rodlphe Brupbacher Prize for Cancer Research. The Advisory Board also suggests Symposia speakers.

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