Charles Rodolphe Brupbacher Stiftung

Breakthroughs in Cancer Research and Therapy

Edited by

Markus G. Manz
Sir Alex Markham
Klaus Rajewsky

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Charles Rodolphe Brupbacher Preis für Krebsforschung 2015

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 2015 wird verliehen an:

Irving L. Weissman, Stanford, USA und
Joan Massagué, New York, USA

Charles Rodolphe Brupbacher Prize for Cancer Research 2015

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 recipients of the 2015 Prize are:

Irving L. Weissman, Stanford, USA and
Joan Massagué, New York, USA

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

Liszt: Au bord d'une source, Années de pèlerinage, première année: Suisse

Laudatio
Irving L. Weissman, M.D.
durch
Prof. Dr. Markus G. Manz

Preisverleihung
Mme Frédérique Brupbacher, Präsidentin der Stiftung

Referat des Preisträgers
Irving L. Weissman, M.D.

Schumann / Liszt: Widmung

Laudatio
Joan Massagué, Ph.D.
durch
Prof. Dr. Paul Kleihues

Preisverleihung
Mme Frédérique Brupbacher, Präsidentin der Stiftung

Referat des Preisträgers
Joan Massagué, Ph.D.

Chopin: Ballade Nr. 1 g-Moll, Op. 23

Schlussworte
Prof. Dr. Klaus W. Grätz

Apéro

Introduction
Prof. Dr. Michael Hengartner, Rector of the University of Zurich
Prof. Dr. Klaus W. Grätz, Dean, President of the Scientific Advisory Board

Liszt: Au bord d'une source, Années de pèlerinage, première année: Suisse

Laudatio
Irving L. Weissman, M.D.
by
Prof. Dr. Markus G. Manz

Award
Mme Frédérique Brupbacher, President of the Foundation

Acceptance Speech
Irving L. Weissman, M.D.

Schumann / Liszt: Widmung

Laudatio
Joan Massagué, Ph.D.
by
Prof. Dr. Paul Kleihues

Award
Mme Frédérique Brupbacher, President of the Foundation

Acceptance Speech
Joan Massagué, Ph.D.

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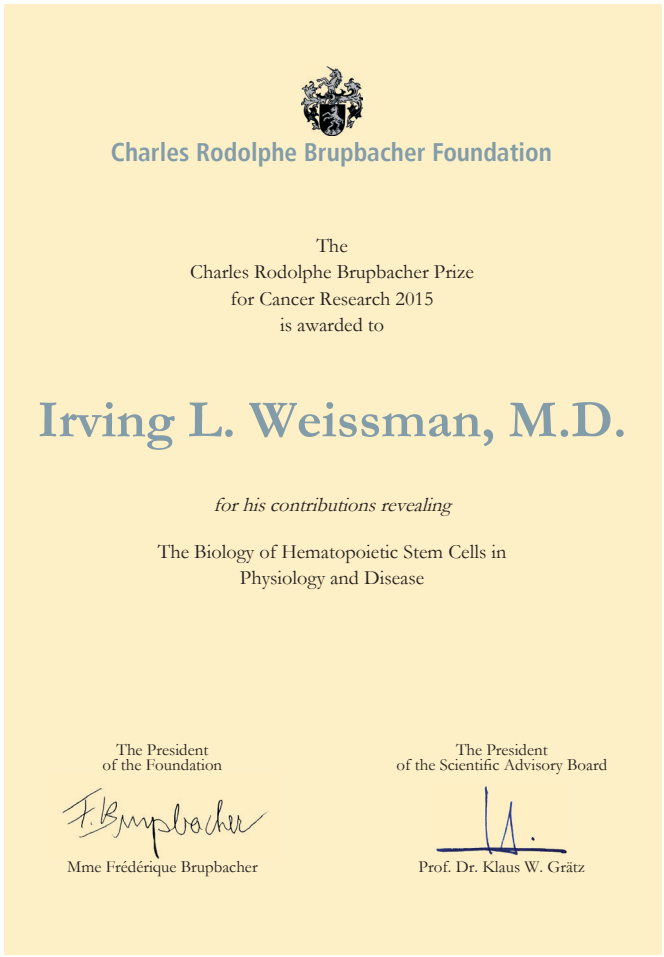
Final address
Prof. Dr. Klaus W. Grätz

Apéro



Laudatio

Markus G. Manz

When I met Irving Weissman almost twenty years ago, I walked in his tiny office at Stanford, packed with paper and magazines, and only realized him when he peeked over a pile, giving me a huge smile and then shouting "Now, who's that?". With my European-trained manners towards medical school authority, this was a first, somewhat shocking confrontation with his joyful curiosity that generically characterizes him. He already was a super-star in both immunology and hematology research, and I was thrilled that he then accepted me as a postdoctoral fellow in his laboratory.

Irving Weissman was born and raised in Great Falls, Montana, did zoology studies at Dartmouth College in Hanover, New Hampshire, pre-med studies at Montana State University in Bozeman, and then studied medicine at Stanford, where, with the exemption of a short stay in Oxford England, he continues to work. After a postdoctoral fellowship with Henry Kaplan at Radiology, he became professor of pathology with subsequently multiple additional appointments (details are listed in the program) and is now leading the Stanford Institute for Stem Cell Biology and Regenerative Medicine as well as the Stanford Ludwig Center for Cancer Stem Cell Research and Medicine. He is recipient of multiple awards (listed in the program) and member of the US American National Academy of Sciences.

Dr. Weissman is publishing since 1957, when he was still a teen-ager (by now over 800 publications of which over 80 appeared in the "big three", Cell, Nature and Science). He is one of the rare species with a very broad interest and the capacity to integrate different fields. While first focusing on transplantation tolerance and lymphoid system development, he then extended his research to hemato-lymphoid cell migration (including metastasis formation) and to stem cells in hematopoiesis, neurogenesis and cancer. Although working mostly on mammals, he also developed a research program in Monterey, California, on Botryllus schlosseri, the beautiful golden star tunicate that grows in saltwater environments, a research leading to bench-mark discoveries of their fascinating model immune system that teaches about self and non-self, and natural transplantation reactions. Also, he is an entrepreneur who founded several companies with the intention to bring scientific achievement to patients. These companies again published great science, however, Irv also discovered that (I cite from a Nat Biotech Interview in 2011) the "…. dilemma, when the medical school want to save lives, and the companies want to make a profit.." needs some new approach, a road he is currently taking with the California Institute for Regenerative Medicine.

Charles Weissmann, a scientist and former Zürich professor known to most of us and tightly connected to the Brupbacher Foundation, at this spot said that "for a professor it is more rewarding to educate students than to write papers because the half-live of papers is about three years while that of students is 30 years". Even if you see him rarely while working with him, Irv Weissman inspires and supports his pupils with his enormous generosity (including an annual lab-retreat on his ranch in Montana). Many of them have accomplished superb own careers in science and medicine and became mentors themselves. In fact, if you

go to international hematopoiesis, stem cell or immunology meetings, you always will find multiple former lab-members being prominently present, and ready for an ad hoc lab-meeting.

To laude Irv Weissman's achievements relevant for the here given award, I try to sketch hemato-lymphopoiesis in a nutshell: The hemato-lymphatic system is a paradigmatic, somatic stem-cell supported organ that serves as a "role-model" for both deciphering physiology and pathophysiology, as well as applying radically new therapeutic approaches. Hematopoietic stem cells (HSCs) are a very rare population of cells in the bone marrow that self-renew, and, through a series of differentiation and expansion steps, can give rise to all mature blood cells throughout the life of an individual. The hematopoietic system is one of the organs of the body with the highest proliferative activity. Amazingly, blood production seems rarely limited by HSC function, at least in young and middle-aged individuals. Indeed, HSCs can be transferred from donors to recipients, and, although only a small fraction of donor HSCs are used, they can expand in the recipient to reach similar homeostatic pool-sizes. However, once HSCs are compromised by either not producing enough offspring (leading to aplasia) or by proliferating uncontrolled (leading to leukemia), the consequences for the individual are massive.

Irv Weissman's groundbreaking identification and isolation of HSCs first in mice in 1988 and subsequently in humans in 1992, followed by deciphering the earliest developmental steps toward lympho- and myelopoieis in both species, his discoveries on aging in stem-cell systems, and finally his contributions to understanding pathways of stem cell to cancer transitions, and the ways the innate immune system might control these neoplastic cells, set the foundation for an enormous, lasting eruption of research and knowledge in the field.

All this and the continuous energy to translate science into better medicine make Irv Weissman a role-model and scientific giant. Congratulations Irv, today this is rewarded with the Charles Rodolphe Brupbacher Foundation Prize for Cancer Research!

Irving L. Weissman

Summary Curriculum vitae

Appointment Institute for Stem Cell Biology and Regenerative Medicine
Stanford University

Address 265 Campus Drive West, Room G3167
Stanford, CA 94305-5461

Stanford University Directorships/Professorships

Director, Stanford Institute for Stem Cell Biology and Regenerative Medicine
Director, Stanford Ludwig Center for Cancer Stem Cell Research and Medicine
Virginia and D. K. Ludwig Professor for Clinical Investigation in Cancer Research
Professor of Pathology, Developmental Biology, and, by courtesy, Biological Sciences and Neurosurgery
Director, Stanford Cancer Center (2005–2008)
Chairman, Stanford University Immunology Program (degree-granting), 1986 – 2001

Education

Stanford University, Stanford - California (Postdoctoral Fellow, H.S. Kaplan)	6/65 – 6/67
Oxford University, Oxford - England (Experimental Pathology)	5/64 – 12/64
Stanford University, Stanford - California (Medicine), M.D., 1965	9/60 – 6/65
Montana State University, Bozeman - Montana (Pre-Med), B.S., 1961	9/59 – 6/60
Dartmouth College, Hanover - New Hampshire (Zoology)	9/57 – 6/59

Fellowships

- Faculty Research Awardee, American Cancer Society (National), 1974 – 1978
- Josiah Macy Foundation Scholar, 1974 – 1975
- Senior Dernham Fellow, California Division of the American Cancer Society (National), 1969 – 1973
- NIH Postdoctoral Fellowship, Department of Radiology, Stanford University School of Medicine, 1965 – 1967
- NIH Student Traineeship, Cellular Immunology Research Unit, MRC, Sir William Dunn School of Pathology, Oxford University, Oxford, England, under Professor J.L. Gowans, F.R.S., Director of Unit, 1964
- NIH Student Traineeship, Department of Radiology, Stanford University School of Medicine, under Dr. Henry S. Kaplan, Professor and Chairman of the Department of Radiology, 1961 – 1964
- Montana Cancer Society Student Research Fellow at the Laboratory for Experimental Medicine, Montana Deaconess Hospital, Great Falls, Montana, under Dr. E. J. Eichwald, Director of Laboratories, Chief Editor, Transplantation Bulletin (now Transplantation), 1956 – 1961

Awards & Honors

2014 Elected, Fellow, American Association for Cancer Research, Philadelphia, Pennsylvania
2013 Award of Honor, The Radiological Society of North America, Chicago, Illinois
2013 Charles Rodolphe Brupbacher Prize for Cancer Research, Charles Rodolphe Brupbacher Foundation, Zurich, Switzerland (notified in 2013 that this will be awarded in 2015)
2013 Alumni Achievement Award, College of Letters & Science, Montana State University, Bozeman, Montana
2013 Agency for Science, Technology and Research (A*Star) National Day Award, The Public Service Medal (Friends of Singapore), A*Star, Republic of Singapore
2013 Max Delbruck Medal of the Max Delbruck Center, Berlin, for research that has a fundamental biomedical impact and a broad interdisciplinary perspective
2012 Hall of Fame, Montana BioScience Alliance, Montana
2012 Bennett J. Cohen Award, University of Michigan, Ann Arbor, Michigan
2011 National Academy of Sciences Council, National Academy of Sciences, Washington, DC
2011 Commencement speaker, PhD graduates, University of Southern California Medical School
2010 President, International Society for Stem Cell Research
2010 Simon M. Shubitz Award for Excellence in the Field of Cancer Research, University of Chicago, Chicago, Illinois
2010 Honorary Investigator, State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, China
2010 Honorary Professor, Peking Union Medical College, China
2009 Honorary Director of the Center for Biotech and BioMedicine and the Shenzhen Key Lab of Gene & Antibody Therapy, Graduate School of Shenzhen, Tsinghau Universit, China
2009 The Cockrell Foundation Award in Clinical or Translational Research, The Methodist Hospital Research Institute, Houston, Texas

2009 Rosenstiel Award, Brandies University, Waltham, Massachusetts (shared with Shinya Yamanaka and John Gurdon)
2009 Passano Award, The Passano Foundation, Baltimore, Maryland
2008 Elected to American Philosophical Society, Philadelphia, Pennsylvania
2008 Fellow, American Association for the Advancement of Sciences, Chicago, Illinois
2008 Robert Koch Award, Koch Foundation, Berlin, Germany (shared with Shinya Yamanaka and Hans Scholer)
2007 Honoree of the Arthritis Foundation of Northern California Chapter's 2007 Tribute Dinner
2007 I. & H. Wachter Award, I. & H. Wachter Foundation
2007 Doctor of Science (Honoris Causa), Mount Sinai School of Medicine, New York City, New York
2006 John Scott Award, City of Philadelphia, Philadelphia, Pennsylvania
2006 American-Italian Cancer Foundation Prize for Scientific Excellence in Medicine, New York City, New York
2006 Honorary Doctorate, Columbia University, New York City, New York
2006 The Commonwealth Club of California 18th Annual Distinguished Citizen Award
2005 Jeffrey Modell "Dare to Dream" Award, Jeffrey Modell Foundation
2005 The Linus Pauling Medal for Outstanding Contributions to Science, Stanford University
2004 New York Academy of Medicine Medal for Distinguished Contributions to Biomedical Research
2004 Alan Cranston Awardee, Alliance for Aging Research
2004 Jessie Stevenson Kovalenko Medal, National Academy of Sciences Council
2004 Rabbi Shai Shacknai Memorial Prize in Immunology and Cancer Research, The Lautenberg Center for General and Tumor Immunology
2003 J. Allyn Taylor International Prize in Medicine
2003 Commencement Speaker to Ph.D. Graduates in Molecular and Cell Biology, University of California, Berkeley
2003 Society of Neurological Surgeons Bass Award
2003 American Diabetes Association Elliott Proctor Joslin Medal
2002 Commencement Speaker to all Graduates (MS, MD, PhD), Stanford University School of Medicine
2002 Election to the Institute of Medicine of the National Academy of Sciences
2002 Basic Cell Research Award by the American Society of Cytopathology
2002 Van Bekkum Stem Cell Award
2002 Association of American Cancer Institutes 2002 Distinguished Scientist Award
2002 California Scientist of the Year
2001 Ellen Browning Scripps Society Medal
2001 Irvington Institute Immunologist of the Year
1999 E. Donnall Thomas Prize to recognize pioneering research achievements in hematology, American Society of Hematology
1999 Leukemia Society of America de Villier's International Achievement Award
1997 Election to the American Academy of Microbiology
1995 Elected Honorary Member, Israel Immunological Society
1994 President, American Association of Immunologists
1994 Montana Conservationist of the Year Award
1993 Selected Top 100 Alumni of Montana State University
1992 Election to the California Academy of Medicine
1992 Honorary Doctor of Science Degree from Montana State University
1990 Election as a Fellow of the American Association for the Advancement of Science
1990 Election to the American Academy of Arts and Sciences
1989 Pasarow Award for Outstanding Contribution to Cancer Biology
1989 The Harvey Lecture
1989 Election to the National Academy of Sciences
1987 Karel and Avice Beekhuis Professor of Cancer Biology
1987 Kaiser Award for Excellence in Preclinical Teaching
1986 Outstanding Investigator Award, National Institutes of Health

Main Research Interests

- Leukemia and cancer stem cells; programmed cell removal pathways
- Hematopoietic stem and progenitor cells
- Central nervous system stem and progenitor cells
- Lymphocyte differentiation
- Homing receptors
- Normal and neoplastic hematolymphoid development
- Phylogeny of stem cells and alloreactivity in protochordates

Research & Professional Experience

- Director, Stanford Institute for Stem Cell Biology and Regenerative Medicine, 2002 – present
- Director, Stanford Ludwig Center for Cancer Stem Cell Research and Medicine, 2007 – present
- Professor, Department of Neurosurgery, Department of Medicine, Stanford University Medical Center, 2004 – present (by courtesy)
- Director, Institute of Cancer/Stem Cell Biology and Regenerative Medicine, 2003 – 2006
- Director Stanford Cancer Center, 2006 – 2010
- Professor, Department of Biology, Stanford University, 1990 – present (by courtesy)
- Professor, Department of Developmental Biology, Stanford University, 1989 – present
- Karel E. Beekhuis Professor of Cancer Biology, 1987 – 2005
- Chairman, Stanford University Immunology Program (degree-granting), 1986 – 2001
- Professor, Department of Pathology, Stanford University, 1981 – present
- Investigator, Howard Hughes Medical Institute, Stanford University, 1990 – 1992
- Associate Professor, Department of Pathology, Stanford University, 1974 – 1981
- Assistant Professor, Department of Pathology, Stanford University, 1969 – 1974
- Research Associate, Department of Radiology, Stanford University, 1967 – 1968
- Elected Member, Steering Committee, Stanford Medical School Faculty Senate
- Elected Member, Stanford University Faculty Senate – President's Office
- Elected Member, Steering Committee [Committee of Five], Stanford University Faculty Senate – President's Office
- Premed Advisor All Chicano and Native American Premeds, Academic Affairs Office, 1968 – 1988
- Member, Stanford Medical Scientist Training Program
- Director, Stanford Medical Scientist Training Program
- Executive Committee, Stanford Medical School

Biotechnology Boards

- Founder and Director, Cellerant, Inc., 2000 – 2010
- Scientific Advisory Board, Fate Therapeutics, 2009–2010
- Co-founder, Stem Cells, Inc., Director, Chair of SAB, 1998 – present
- Member, Board of Directors, SyStemix, 1988 – 1997
- Chairman, Scientific Advisory Board, SyStemix, 1988 – 1997
- Co-founder, SyStemix, 1988
- Scientific Advisory Board, (Founding) T Cell Sciences, 1988 – 1992
- Scientific Advisory Board, (Founding) DNAX, 1981 – 1992
- Scientific Advisory Board (Founding), Amgen, 1981 – 1989

The view from stem cell land: Stem cell biology in regeneration and cancer

Irving Weissman

In 1961 as a first year medical student at Stanford University, enrolled in a medical school that provided nearly 40% of our time during all 5 years of medical school to pursue scholarly and scientific research, I bumped into Henry Kaplan, a leading physician-scientist who had provided me with a lab and the example of how to translate discoveries to medical advances. He showed me a paper written by James Till and Ernest McCulloch in the journal Radiation Research that demonstrated that bone marrow transplants at limiting numbers into lethally irradiated mice led to a cell dose-dependent formation of donor cell colonies, each of which had several cells in the monocyte, granulocyte, megakaryocytic, and erythroid lineages. While Till and McCulloch were cautious at that time not to call the colony forming cells blood-forming, or hematopoietic stem cells [HSC], that was the beginning of the field of stem cell biology.

Over 25 years later my group developed the method that isolated blood-forming stem cells in mice and humans, and then began the process to identify and isolate each stage of development from stem cell to blood cell. In our earliest experiments we showed that mouse HSC were the only active cells in bone marrow transplants that led to rapid and sustained regeneration of the blood forming system in irradiated mice. Human HSC, as we isolated them were the only cells in the tube, and therefore were essentially free of the contaminating cancer cells found in blood forming tissues of people with metastatic breast cancers or aggressive lymphomas.

We therefore moved rapidly at a company I co-founded, SyStemix, to test whether cancer-free HSC taken from a patient could be used to regenerate the blood forming system of patients receiving potentially lethal doses of combination chemotherapy, the higher doses given to eliminate even more cancer cells in the body. Although even our early results in progression-free survival of these patients were much better than restoring patients with the unmodified bone marrow or mobilized blood that was the standard of care at the time, the large pharma that bought SyStemix decided to close down the program mid-trial. . When a decade and a half later, years after the trial was complete we checked the results from women with stage IV metastatic breast cancer treated at Stanford with either cancer-free HSC or cancer-contaminated mobilized blood, at 12-15 years post-transplant 33% of the patients receiving cancer-free HSC were alive vs only 7% of those receiving mobilized blood.

The closure of the program by the pharma also brought to a halt the regenerative medicine program we had established in SyStemix, to use pure donor HSC, free of contaminating donor T cells, to regenerate the system of recipients without causing a graft vs host disease from the immunocompetent T cells in the donor graft. We planned to replace the host defective hematopoietic cells that were the cause of deficiency diseases such as severe combined immune deficiency [SCID], sickle cell and Mediterranean anemias, and genetic autoimmune disorders such as lupus, type 1 diabetes, and multiple sclerosis. We had already shown in mice that the pure HSC, lacking T cells, engrafted but did not cause graft vs host disease, the constant side affect of such transplants that causes morbidity and mortality and is countered only by lifelong immunosuppression. We had also shown that any organ or tissue graft from the HSC donor was accepted with no requirement for immunosuppression other than for conditioning of the host to accept the HSC. We had also shown that HSC from donor mice cured SCID, and if given early enough from donors lacking the genetic predilection for autoimmune diseases, prevented progression to type 1 diabetes, or to death by renal failure in lupus mice. Late stage diabetic NOD mice could be cured with a co-transplant of HSC and insulin-producing cells from the HSC donor.

One problem with HSC transplants for regenerative medicine is the necessity, currently, to take the patients close to mortality by the drugs and irradiation used to make space for new HSC, and to eliminate the immune reactive T cells and NK cells that reject allografts. In the past 15 years we have found antibodies to T cells, and in mice to NK cells, that remove the need for the immunosuppressive properties of these conditioning regimens. Recently we have found antibodies that selectively deplete HSC and their early progenitors, and these enable mice with SCID to be saved with T cell free healthy MHC matched HSC. We have found an antibody counterpart for humans, and have current funding to carry out T cell free HSC transplants into SCID children [who have lost their grafts or have no suitable donor] with antibody conditioning. This line of investigation could lead to a new era of regenerative medicine in which patients are conditioned with antibodies, probably in the outpatient setting, and transplanted with HSC from a living donor who could also be an organ donor, or in the more distant future when both HSC and tissue stem cells derived from

the same pluripotent stem cell line [ES or iPS] could be co-transplanted into HLA matched patients.

When we had finally identified and isolated mouse HSC and the cells that made up the progenitors for blood, it became clear that only HSC of all of these cells could self-renew. This came at a time when we and others were finding oncogenes or cancer-enabling or promoting genes that could not endow self-renewal. John Dick has proposed that in human acute myelogenous leukemias [AML] a leukemia stem cell [LSC] existed that shared properties with HSC, and he suggested could be HSC. That would allow genes that don't cause self-renewal to accumulate in HSC that were in transit to becoming LSC. However, in the late 1990s we found that mouse AML LSCs were at the granulocyte-macrophage progenitor [GMP] stage, and that LSC from a particular kind of human AML were at the multipotent progenitor [MPP] stage, one or a few stages below HSC, cells that usually can't self-renew and that fail in bone marrow transplants. So how did the genes that don't cause self-renewal appear in human AMLs? The clue came from finding a particular genetic change, called a chromosomal translocation wherein two different chromosomes break apart, and can re-join with the wrong partner chromosome. The joining point can make an oncogene, such as bcr to abl in chronic myelogenous leukemia, or in our case, with the leukemias we studied, joining aml1 to eto to make a fusion gene, aml1-eto. While the particular unique translocation [which had to begin in a single cell] was found in every MPP LSC in the patient, the same translocation was in a small percent of normal HSC, and these cells were normal in making all kinds of blood without making leukemias. We proposed that the mutations or inherited changes in gene expression that play roles in cancer development, here leukemia development, but did not encode the property of self-renwal, had to occur in a 'clone' of HSC derived from the first mutation in a single HSC, that could accrue the other mutations or altered gene expressions one at a time over a very long time interval. By identifying all of the mutations in the leukemia cells of a particular patient, we could prepare DNA probes specific for each mutation in that patient. We then isolated from the patient HSC, and put one HSC per well in a culture dish. We could then give factors to the HSC that make it grow and differentiate to MPP and GMP and the other progenitors, and then test each 'clone' with the entire set of DNA probes. We found that we could find many HSC with no mutations, some with only mutation 1, others with mutations w and 2 [but none with 2 alone], and others with 1 and 2 and 3, etc. We had shown the order of mutations for each leukemia, that the mutations established a clone of HSC that self-renew and gradually acquire more mutations, and that the entire process except for the last mutation occurred in HSC –like self renewing cells. The last mutation occurred in the MPP stage for several independent AMLs, and the mutations, e.g. flte internal tandem repeat or K-ras give massive proliferative capacity to the clone, hence, leukemia. We found that in chronic myelogenous leukemia [CML] the early mutation, translocation of bcr to abl, occurs in HSC, causing a very slowly progressive proliferative disorder. When the CML abruptly takes off and will kill the patient in weeks, the HSC clone with bcr-abl gave rise to a GMP that had turned on a highly proliferative gene called beta-catenin, a gene whose activity drives HSC and brain stem cells and intestinal stem cells, to name a few. So in CML progression, as in AML, early mutations prepare the cell to survive proliferation and host surveillance, and later mutations drive the cell to proliferate massively and overgrow the original organ in which the cells reside. But what about host surveillance to guard against cancer development?

This answer came from a parallel set of studies we made with blood cells. In the 1980s it became apparent the programmed cell death [PCD] of aberrant of infected cells was a property that cancer cells somehow evaded. A cell death pathway, in fact several cell death pathways were revealed; e.g., aberrant cells can signal through p53, a protein that is turned on by many alterations within a cell that could compromise the cell's functions. Complete loss of p53 expression is a hallmark of many cancers, and so we now call p53 a tumor suppressor gene. Other cells can gain expression of bcl2 or related genes, whose function is to block PCD in cells that need it, but which can be a positive stimulus to cancer, and is called an oncogene or protooncogene. To study bcl2 in the blood forming system we made mice that overexpressed bcl2 only in the GMP and its progeny white blood cells. One type of white cell, the neutrophile, normally had a 1-2 day lifespan in the body, and that didn't increase with bcl2 expression, even though these cells didn't die over many days in a test tube by themselves. But in the body they disappeared at a rate that their numbers never increased in the blood or tissues. We then found that both about to die neutrophiles [about 12 hours old from their birthdate]and the deathless bcl2 neutrophiles put on their surface 'eat me' signals for roaming scavenger cells called macrophages. So bcl2 blocked PCD, but didn't block programmed cell removal [PrCR]. The property of PrCr in dying cells allows them to finish the dying process inside of macrophages instead of out in the tissues, where their death would cause inflammation[coined as 'death induced inflammation' by a

former Brupbacher awardee, Michael Karin]. PrCR therefore appeared to be a process to prevent death induced inflammation. This all became clearer when in 1998 David Traver and I looked at genes expressed in mouse LSC but not normal mouse GMP; of the many overexpresses genes , the highest change was in a gene called CD47. In 2000 Oldenberg and Lindberg published that CD47 was an age marker on mouse red blood cells, working by acting as a ligand for a macrophage receptor called Sirp.a. Binding CD47 to Sirp.a temporarily paralyzes the macrophage so that it can't eat the cell to which it is attached as it scans blood and tissues for cells with eat me signals. So a 'don't eat me' signal comes up in all mouse leukemias. When we found that human AMLs also selectively expressed more CD47 than normal MPPs or HSCs, it was time to see how 'don't eat me' plays a role in cancer development. In a large series of experiments using patient primary solid tumors or AML or lymphomas we could show that all overexpressed CD47, that the leukemias and lymphomas at least co-expressed a protein on their surface called calreticulin, which is an 'eat me ' signal for macrophages, and that blocking CD47 with antibodies caused macrophages to eat the leukemia cells, and blocking both CD47 and calretiulin on the same cell with antibodies resulted in no eating of the leukemia cells by macrophages. The anti-CD47 blocking antibody enabled all human cancer cells to be eaten in a petri dish by human macrophages, and we therefore wondered if it could be a cancer therapeutic. We found that all human solid tumors, leukemias, lymphomas, etc. that transplant into mice that lack the T cell, B cells, and natural killer cells that would reject human tissues in mice could grow until the mouse was infused with the anti-human CD47 blocking antibody; the antibody led to massive tumor phagocytosis and death inside macrophages, sometime curing the mouse, sometimes just slowing the tumor down, but in all cases eliminating the seeds of tumors at distant sites called metastases. The antibodies that block the don't eat me CD47 synergized with other anticancer antibodies that improve phagocytosis, so that anti-CD47 plus rituximab cures most malignant lymphomas growing in mice, that anti-CD47 plus Herceptin synergizes to eliminate the most aggressive her2+ breast cancers growing in immune deficient mice, etc. With the help of funding from the Ludwig Institute and from the California Institute of Regenerative Medicine our university team has made and 'humanized' our best anti-CD47 blocking antibody, done the types of toxicity testing and antibody dosing that usually happens in biotechs or pharma, and we have submitted an IND application to the US Federal Drug Administration, were approved to begin clinical trials, and they have begun at Stanford.

This is where we are, largely, in tracking stem cells and cancer stem cells, leaving out other work the lab does. We have brought together strands of data and observation on stemness, stem cell self-renewal, precancerous progression in tissue stem cells, and the phenomenon of PrCR and its blockade in all cancers to clinical translation, and we are trying to keep it in the university long enough so that no pharma or biotech licensee can close it down as the less favorable commercial opportunity.

References:

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Lagasse E, Weissman IL (1994): bcl-2 inhibits apoptosis of neutrophils but not their engulfment by macrophages. J Exp Med 179:1047–52.

Weissman IL (2000): Translating stem and progenitor cell biology to the clinic: barriers and opportunities. Science. 287:1442–6.

Uchida N et al (2000): Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci U S A. 97:14720–5.

Reya T et al (2001): Stem cells, cancer, and cancer stem cells. Nature. 414:105–11.

Weissman IL (2002): Stem cells − scientific, medical, and political issues. N Engl J Med 346:1576–9.

Jamieson CH et al (2004): Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med 351: 657–67.

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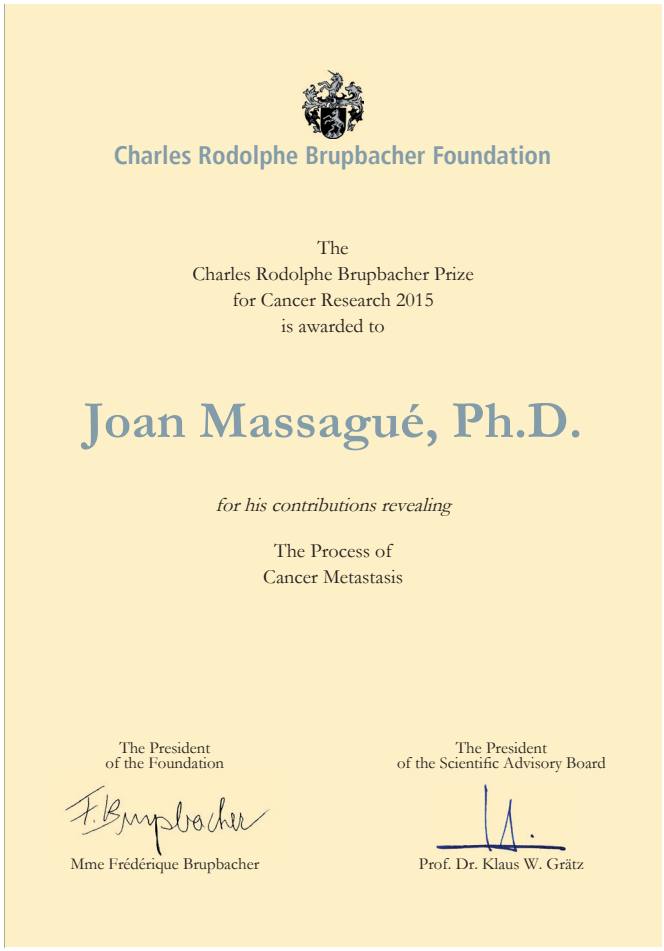

Laudatio

Paul Kleihues

Metastatic spread to distant organs is generally the worst prognostic factor for the survival of cancer patients. Owing to their critical clinical significance, the mechanism and targeting of metastasis have been studied for a long time, but are not well understood. Glioblastoma, the most malignant tumour of the nervous system, infiltrates the brain so diffusely that successful surgical resection is not possible. Nevertheless, glioblastoma cells are unable to cross the pia mater into the subarachnoidal space, neither do they manage to cross the blood-brain barrier and spread to other organs. Paradoxically, some tumour types readily metastasize to the nervous system, particularly breast and lung cancer, but also including the less frequently-occurring clear cell renal cell carcinoma and melanoma. Circulating breast and prostate cancer cells preferentially target bones, but prostate cancer does not spread to the nervous system. While some metastatic patterns reflect lymphatic and blood circulatory pathways, most of them do not.

Enter Dr. Juan Massagué, who used elaborate genetic and epigenetic analyses to understand some of these patterns of enigmatic site-specific metastasis.

As early as 2003, he reported that human breast cancer cell lines which generate osteolytic bone metastases with high efficiency are characterized by expression of three cooperating genes. These encode osteolytic and angiogeneic factors, and the pro-metastatic transforming growth factor beta. This key publication, cited by other authors more than one thousand times, also showed that the metastatic process requires the involvement of genes that may not have been essential in the development of the primary tumour. More recently, Dr Massagué and his team showed that the stroma of primary tumours can be a determining factor in the context of metastatic targeting. The proteins fCXCL12 and IGF1, derived from cancer-associated fibroblasts, can drive triple-negative breast carcinoma cell populations toward enrichment for clones that have a constitutively high level of Src activity and a propensity for metastatic growth in bone.

In his laboratory, by comparing the pattern of gene expression in human breast cancer cell lines with low and high potential to generate lung metastases, Dr. Massagué clearly identified a set of genes which mediate metastasis to the lung, and at the same time promote growth of the primary breast carcinoma. The lung metastasis expression signature in breast carcinomas appeared to independently predict spread to the lung and a poor prognosis overall.

Using mouse xenograft models, his team recently showed that high expression of the lysosomal enzyme cathepsin S greatly facilitates breast-to-brain metastasis. However, only about one in a thousand circulating breast or lung cancer cells that managed to enter the brain were able to survive. The others were killed by astrocytes that secrete Fas ligand and the protease plasmin converts membrane-bound Fas ligand into a paracrine signal which triggers apoptosis, an internal suicide program. Dr. Massagué and his colleagues were also able to identify a survival mechanism: the few carcinoma cells escaping apoptosis over-expressed serpin proteins, which counteract plasmin.

Clear cell renal cell carcinomas are the most common malignant neoplasm of the kidney in adults. They typically spread to the lung via the vena cava, but they also spread to unusual sites, including the brain, sometimes several years after resection of the primary neoplasm. Up to 70% of these tumours are attributable to inactivation of the von-Hippel-Lindau gene by mutation, allelic deletion or epigenetic silencing. The von-Hippel-Lindau gene product, normally specified as VHL, causes degradation of the hypoxia inducible factor HIF2 α that activates the expression of several genes with tumorigenic and pro-metastatic functions. Dr. Massagué has shown that DNA methylation restricts expression of the metastasis-associated VHL-HIF target genes and thereby reduces the capacity of affected cells to spread to other organs. This demonstrates that in some cancers, including clear cell renal cell carcinoma, development of the capacity to metastasize becomes operative during progression along the tumour-initiating pathway. Hence the metastatic cascade may be less dependent on metastasis-promoting driver mutations in the primary tumour than on epigenetic stimulation of cell survival and self-renewal mechanisms.

These are just a few of the many examples of Dr. Massagué's scientific discoveries that have greatly influenced the field of organ-specific metastatic spread. Identifying the genes responsible for initiation and completion of the metastatic process may allow development of strategies to decrease or abolish the capacity of circulating tumour cells to form metastases in distant organs.

In recent years, evidence has emerged that the two main themes of this year's Symposium – cancer stem cells and mechanisms of metastasis – are tightly linked biologically. Dr. Massagué and colleagues have emphatically shown that phenotypic properties and signalling pathways of disseminated cancer cells that enable metastatic growth in distant organs overlap significantly with those of normal stem cells. The chance that a cancer cell will emigrate from a primary neoplasm to form a metastasis is incredibly low. There are, however, shelters in which these cells may survive. One such place is the bone marrow, a classical site where tumour stem cells can hide and from which they may eventually migrate and successfully colonize other tissues. In very elegant studies, Dr. Massagués team showed that in the brain there is a perivascular niche that greatly facilitates colonization. Metastasis-initiating cancer cells, after overcoming the blood-brain barrier, stick to and grow on the surface of blood capillaries. This interaction with capillaries ensures a generous supply of oxygen and glucose, which enables the formation of a multi-layered perivascular sheath from which surviving cells eventually form a metastasis.

Throughout his scientific career, Dr. Massagué has been scientifically productive and innovative. His work on mechanisms of metastasis has finally opened the door to a better understanding of the fundamentals of the metastatic process and we look forward to more discoveries from his laboratory, which we hope will lead to metastasis-preventing treatments. On behalf of the Charles Rodolphe Brupbacher Foundation and all of us at this exciting symposium, sincere congratulations to Dr. Massagué as recipient of the Brupbacher Award 2015 !

Joan Massagué

Summary Curriculum vitae

Appointment Director, Sloan Kettering Institute

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Date of Birth April 30, 1953.

Education

1975 B.S., University of Barcelona
1978 PhD, Biochemistry, University of Barcelona.

Professional Appointments

1979–82 Research Fellow, Brown University.
1982–85 Assistant Professor of Biochemistry,
U. Massachusetts Medical School
1985–89 Associate Professor of Biochemistry,
U. Massachusetts Medical School
1989– Alfred P. Sloan Chair, Memorial Sloan Kettering
Cancer Center
1989–13 Chairman, Cell Biology and Cancer Biology
Programs, MSKCC
1990–13 Investigator, Howard Hughes Medical Institute
2014– Director, Sloan Kettering Institute
2014– Provost, Gerstner Sloan Kettering Graduate School
of Biomedical Sciences

Advisory Boards (partial list)

1996–00 Board of Scientific Advisors,
National Cancer Institute
1998–10 External Advisory Board,
MD Anderson Cancer Center
2000–14 Member and Chair,
Scientific Advisory Board, CNIO, Madrid
2009–12 Board of Directors, American Association for
Cancer Research
2006– Adjunct Director; External Advisory Board Institute
for Research in Biomedicine Barcelona
2010– Board of Trustees, The Vilcek Foundation
2014– Chair, External Advisory Board, Institute for
Research in Biomedicine Barcelona
2015– Scientific Review Board,
Howard Hughes Medical Institute

Editorial Boards (partial list)

Proceedings of the National Academy of Sciences USA;
Cell; Genes & Development; EMBO Journal;
EMBO Molecular Medicine; Journal of Clinical Investigation;
Journal of Cell Biology; Cancer Discovery; eLife

Awards And Honors (partial list)

1979 Fulbright Foundation Postdoctoral Fellowship
1993 King Juan Carlos I Research Prize
1998 Member, European Molecular Biology Organization
1999 Member, American Academy of Arts and Sciences
2000 Member, National Academy of Sciences, USA
2004 Member, Royal Academy of Medicine of Spain
2004 Prince of Asturias Award in Science and Technology
2005 Member, Royal Academy of Pharmacy of Spain

2006 Member, Institute of Medicine, USA
2006 Vilcek Prize
2007 Passano Laureate Prize
2008 Frontiers Prize in Biomedicine, BBVA Foundation
2009 G.H.A. Clowes Memorial Award, American Association for Cancer Research
2010 Feodor Lynen Medal, Nature-Miami Winter Symposia
2011 Robert J. and Claire Pasarow Prize in Cancer Research
2011 Breast Cancer Innovator Award, Department of Defense
2014 National Prize of Culture, Catalonia
2014 National Prize for Research, Spain

Publications (partial list)

Wrana, J.L., Attisano, L., Wieser, R., Ventura, F. and Massagué, J., Mechanism of activation of the TGF-beta receptor. Nature 370, 341-347 (1994)

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Kim, M.-Y., Oskarsson, T., Acharyya, S., Nguyen, D.X., Zhang, X H.-F., Norton, L., and Massagué, J. Tumor self-seeding by circulating cancer cells. Cell 139, 1315-1326 (2009)

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Valiente, M., Obenauf, A.C., Jin, X., Chen, Q., Zhang, X.H.F., Lee, D.J., Chaft, J.E., Kris, M.G., Huse, J.T., Brogi, E. and Massagué, J. Serpins promote cancer cell survival and vascular cooption in brain metastasis. Cell 156, 1002-1016 (2014)

Molecular Basis of Metastasis

Joan Massagué

Metastasis, the process by which cancer cells from a primary tumor infiltrate and overtake distant organs, is a central obstacle in oncology today. It would be difficult to overstate the medical and societal importance of this problem. Approximately 90% of deaths from cancer are directly caused by metastasis, not by the primary tumor. A diagnosis of cancer is typically followed by resection of the primary tumor, but by then spread of cancer cells to distant organs may already have occurred. Despite treatment advances to eradicate the residual disease, current clinical management of overt metastasis rarely results in a cure. The mechanisms underlying metastasis must therefore be understood and mined for effective therapies to advance cancer treatment beyond this roadblock.

Metastasis is an old problem. The spreading capacity of malignant tumors earned this disease its name over two millennia ago. Hippocrates called it karkinos (crab, in Greek) in reference to the projections spreading from the tumor like legs from a crab's body. By the 19th century metastasis was recognized as the result of tumor seeds that spread cancer to other organs. In the 1970s Isaiah Fidler and others described many of the basic features of metastasis, including the different profile of affected organs in different types of cancer, the role of the circulation in metastatic dissemination, and the varying metastatic capacity of cancer cells in a heterogeneous tumor. Up until the late 1990s, however, our knowledge about the molecular basis of metastasis remained descriptive in nature. Mechanistic insights were limited to how cancer cells migrate and invade surrounding tissues. While invasion and migration are necessary early steps for tumor dissemination, the vast majority of cancer cells that leave a tumor perish. The mechanisms allowing cancer cells to survive as metastatic seeds and colonize distant organs remained a mystery. The daunting complexity of the metastasis and the lack of appropriate research tools deterred work on the problem.

Three developments at the turn of this millennium paved the way for more research: One was the epic success pioneered by Michael Bishop and Harold Varmus in the 1970s, and joined by many others, to identify genes and molecular pathways that initiate tumor formation. This progress suggested that metastasis eventually could also be dissected and understood at the molecular level. The second was the recognition, eloquently highlighted by Robert Weinberg and Douglas Hanahan, that primary tumors contain a large presence of non-cancerous stromal cells that supports the growth of the tumor. This suggested that the tumor microenvironment could also play a role in selecting for metastatic traits during the clonal evolution of tumor cell populations. The third development was the advent of new technologies, including genome sequencing, gene expression analysis, and in vivo cell imaging that provided key tools to investigate metastasis. These three advances converged to make a mechanistic assault on metastasis possible.

Within this context, lessons learned from dissecting the TGFβ signal transduction pathway turned my attention to metastasis. The transforming growth factor β (TGFβ) pathway plays crucial roles in embryonic development, tissue regeneration, and immunity. Its malfunctions cause congenital disorders, chronic inflammation, fibrotic diseases, and cancer. Earlier, we identified the TGFβ receptors, their signaling mechanism, and the central concept of how this pathway regulates gene expression to control cell proliferation and fate. Of particular interest to us was the paradoxical role of TGFβ in cancer. TGFβ acts as both a suppressor of tumor initiation and a promoter of metastasis. To form tumors, cancer cells must avoid the anti-tumor effect of TGFβ by losing cell death responses to this factor. With this done, cancer cells are free to use TGFβ for metastasis. Asking how so, we found that in breast tumors TGFβ from the stroma stimulates cancer cells to produce angiopoietin-like 4. Educated in this manner, cancer cells can leave the tumor, lodge in lung capillaries and use angiopoietin-like 4 to break out of blood vessels into the tissue. The resulting accumulation of metastatic seeds in the lungs increases the risk of metastasis. These observations taught us that metastatic cells are selected to make use of anything that helps them pass through barriers. We postulated that metastatic cells must have organ-specific traits that are selected during the colonization of different organs. Based on this notion, and using mice as cell sorters, we isolated cancer cells with different organ tropisms from heterogeneous tumor populations of the same patient. These experimental models combined with data from large sets of human clinical samples led to the identification of many genes in breast, lung, and renal cancers that promote or suppress metastasis to the bones, the lungs, or the brain. Some of these genes encode proteins, others micro-RNAs. Their analysis in model systems uncovered unsuspected mechanisms of cancer cell infiltration of distinct organs, survival in the new host tissue, and unbridled outgrowth of metastatic colonies. Notably, these genes not only mediated organ-

specific metastasis in experimental models but predicted relapse to these organs in patients.

These findings foster an intensely Darwinian view of the metastatic process. Metastasis appears as the end result of a selection process in which cancer cells progress through highly demanding steps. Although circulation patterns matter somewhat, cancer cells in the circulation can reach all the organs of the body. No organ microenvironment is favorable to incoming cancer cells. All organs are hostile, some just a little less so than others. Each step in the metastatic sequence is a narrow bottleneck that causes the demise of the vast majority (>99%) of the cancer cells engaged in the process. Each step selects for cells that, though epigenetic changes more than through specific mutations, have gained a higher probability of succeeding. No single gene mutation or deviant mechanism could help cancer cells navigate the entire ordeal. Rather, the selected cancer cells resort to different gene products that in combination increase the probability of successful passage through a particular step. The combination of these cell-autonomous and environmental determinants dictates the organ distribution and the efficacy of metastasis in a given cancer.

Three additional revelations punctuated our work on the molecular basis of metastasis. First, disseminated cancer cells depend on mechanisms that selectively amplify survival and stemness pathways in microenvironment-dependent manner. This phenomenon is manifest in the survival of VCAM1-positive breast cancer cells that profitably interact with leukocytes in the lung parenchyma, or the survival of SRC-positive breast cancer cells that interact with CXCL12-rich mesenchymal cells in the bone marrow. Similarly, breast cancer cells can support their tumor-initiating capacity by producing the extracellular matrix protein tenascin-C, a protein of stem cell niches that enhances Notch and Wnt signaling in metastasis-initiating cells. Moreover, cancer cells can resist the stresses of metastasis and chemotherapy alike by engaging myeloid cells via a TNF-CXCL1-S100A8/9 paracrine loop.

Another unexpected revelation was the phenomenon of tumor self-seeding, whereby previously disseminated metastatic cells can re-infiltrate the tumor of origin. Aggressive clones that leave a tumor and survive in distant organs can re-enter the circulation, re-infiltrate the tumor or origin and expand in this supportive microenvironment. A result of this dynamic cycle is the amplification of aggressive metastatic populations in the primary tumor or in the inflamed parenchyma after tumor removal. Tumor self-seeding provides a plausible explanation for various clinical manifestations of metastasis, response to therapy, or resistance to it. Therefore interference with this process provides an opportunity for therapy against tumor recurrence.

The diversity of metastatic mediators discovered over the past decade underscores the complex biology of this process and, at the same time, raises sobering questions about the possibility of developing drugs to combat metastasis in multiple organs and in different types of cancer. However, recent findings offer hope in this regard. We found that disseminated cancer cells need to adhere to the external surface of blood capillaries, coopting the vessels in order to survive and initiate metastatic growth. To this end, cancer cells express the cell adhesion molecule L1CAM. L1CAM is normally expressed in developing neurons for neurite extension and synapse formation during brain development. Prior reports noted the anomalous expression of L1CAM in different types of cancer as an indicator of poor prognosis. Our unexpected finding that L1CAM mediates vascular cooption by metastatic cells for colony outgrowth points at a general mediator of metastasis at a variety of sites that could be targeted therapeutically.

I hope that this brief review shows that the progress to date has debunked the old myth of metastasis being too difficult to dissect molecularly or deconstruct conceptually. Indeed, the field at large has now embraced the task of taking this problem to the next level of molecular definition, and towards more effective treatments. For example, we are now poised to make progress on the most ominous complication of cancer: brain metastasis. Brain metastasis is highly disruptive and lethal. It is estimated to affect nearly 400,000 people in the US and Europe alone, and is on the rise. Clearly more work on brain metastasis is in order. Finally, latent metastasis –the state of disseminated cancer cells that have not yet initiated aggressive outgrowth– represents a major untapped opportunity to prevent metastasis. By learning more about the mechanisms that sustain latent metastatic cells in hiding and oblivious to drugs that only kill growing cells, we should be able to increase the efficacy of adjuvant therapies to rid the patient's body of residual disease after the removal of a primary tumor. Given the recent rapid progress in our mechanistic understanding of metastasis, I have no doubt that problems such as the secrets of latent disease or the nature of brain metastasis will be conquered.

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Acharyya S et al (2012): A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell 150:165-–78.

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Zhang X H-F et al (2013): Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. Cell 154:1060–73.

Valiente M et al (2014): Serpins promote cancer cell survival and vascular cooption in brain metastasis. Cell 156:1002–16.

Massagué J (2012): TGFβ signaling in context. Nature Rev Mol Cell Biol 18:5521–25.

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Oskarsson T et al (2014): Metastatic stem cells. Cell Stem Cell 14:306–21.

Bisherige Preisträger
Previous Laureates

1993

Arnold J. LEVINE
Department of Molecular Biology, Lewis Thomas Laboratory, Princeton University, Princeton, NJ, USA
«Functions of the p53 Gene and Protein»

David P. LANE
Cancer Research Campaign Laboratories, Department of Biochemistry, University of Dundee, Dundee, Scotland
«The p53 Pathway, Past and Future»

1995

Alfred G. KNUDSON
Fox Chase Cancer Center, Philadelphia, PA, USA
«Hereditary Cancer»

Robert A. WEINBERG
Whitehead Institute for Biomedical Research, Department of Biology, MIT, Cambridge, MA, USA
«Genes and Cancer»

1997

Laurent DEGOS
Institut Universitaire d'Hématologie, Hôpital Saint Louis, Paris, France
«Differentiation Therapy of Cancer»

Zhen-yi WANG
Shanghai Institute of Hematology, Rui-Jin Hospital Shanghai, Second Medical University, Shanghai, China
«Treatment of Acute Promyelocytic Leukemia with All-Trans Retinoic Acid. A Model of Differentiation Therapy in Cancer»

1999

George KLEIN
Microbiology and Tumor Biology Center (MTC)
Karolinska Institute, Stockholm, Sweden
«Cancer and the New Biology»

Harald ZUR HAUSEN
Deutsches Krebsforschungzentrum, Heidelberg, Germany
«Cancer Causation by Viruses»

2001

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

2003

Rudolf JAENISCH
Whitehead Institute for Biomedical Research, Department of Biology, MIT, Cambridge, MA, USA
«Nuclear Cloning and the Reversibility of Cancer»

Erwin F. WAGNER
Institute of Molecular Pathology, Vienna, Austria
«Unravelling the Functions of AP-1 (Fos/Jun) in Mouse Development and Disease»

2005

Mariano BARBACID
Centro Nacional de Investigaciones Oncológicas, Madrid, Spain
«The Molecular Bases of Human Cancer: a 25 Year Journey»

Klaus RAJEWSKY
The CBR Institute for Biomedical Research,
Harvard Medical School, Boston, MA, USA
«The Janus Face of Antibody Formation: Protective Function and Tumor Risk»

2007

Lloyd J. OLD
Ludwig Institute for Cancer Research, New York, NY, USA
«Contributions to the Field of Cancer Immunology»

Robert D. SCHREIBER
Department of Pathology and Immunology, Washington University School of Medicine, St.Louis, MO, USA
«Cancer Immunoediting: Deciphering the Complex Interaction Between Immunity and Developing Tumors»

Mark J. SMYTH
Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
«Extrinsic Tumor Suppression by Innate and Adaptive Immunity»

2009

Nubia MUÑOZ
National Cancer Institute, Bogota, Colombia
«From Causality to Prevention: The Case of Cervical Cancer»

Sir Richard PETO
Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom
«The Absolute Benefits of Anti-cancer Drugs and of Tobacco Control»

2011

Jan HOEIJMAKERS
Department of Genetics, Erasmus Medical Center, Rotterdam, The Netherlands
«DNA Damage and its Impact on Cancer, Aging and Longevity»

Bert VOGELSTEIN
The Sidney Kimmel Comprehensive Cancer Center
Johns Hopkins University, Baltimore, MD, USA
«The Genetic Basis of Human Cancer and its Implications for Patient Management»

2013

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

Programm des Wissenschaftlichen Symposiums 2015

Program of the Scientific Symposium 2015

Charles Rodolphe Brupbacher Symposium 2015
Breakthroughs in Cancer Research and Therapy

Wednesday, January 28, 2015

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

13:30 – 15:00 **Welcome: Markus G. Manz / Klaus Rajewsky**
Sir Alex Markham

Microbiota and Cancer
Chair: Karin Mölling, Berlin

Richard A. Flavell, New Haven
Humanized mice for cancer research

Laurence Zitvogel, Villejuif / Paris
Role of gut microbiota in the efficacy of anticancer compounds

Romina S. Goldszmid, Bethesda
Microbiota and response to cancer therapy

15:00 – 15:30 *Coffee break*

15:30 – 17:30 **Microenvironment and Cancer**
Chair: Sir Alex Markham, Leeds

M. Mark Taketo, Kyoto
Molecular mechanisms of colon cancer metastasis: toward clinical applications

Douglas Hanahan, Lausanne
Therapeutic targeting of the tumor microenvironment

Ingo Ringshausen, Cambridge
Targeting the host cells in B-cell lymphoma - Is it superior to chemotherapy?

Mikala Egeblad, Cold Spring Harbor
Seeing cancer in context:
imaging reveals contributions of the microenvironment to therapy resistance and metastasis

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

Josef Jiricny, Zurich
Personalisierte Krebsmedizin

Thursday, January 29, 2015

08:00 – 08:30 *Registration*

08:15 – 09:30 **Cancer Genetics**
Chair: Klaus Rajewsky, Berlin
Antibody engineering and somatic hypermutation.
A tribute to Michael Neuberger

Sir Michael R. Stratton, Cambridge
Mutational processes in human cancer

Peter A. Jones, Grand Rapids
The cancer epigenome

09:30 – 10:30 **Cancer Stem Cells (1)**
Chair: Lukas Sommer, Zurich

Riccardo Dalla-Favera, New York
Molecular genetics of diffuse large B-cell lymphoma

Anton Berns, Amsterdam
Tumor heterogeneity and cell-of-origin of
mouse small cell and non-small cell lung cancer

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

11:00 – 12:30 **Cancer Stem Cells (2)**
Chair: Markus G. Manz, Zurich

Elaine Fuchs, New York
Stem cells in silence, action and cancer

Walter Birchmeier, Berlin
Wnt / beta-catenin and cooperative signaling sytems in cancer stem cells

Irving L. Weissman, Stanford
Normal and neoplastic stem cells

12:30 – 14:00 *Lunch + Coffee + Posters*

14:00 – 15:30 **Mechanisms of Metastasis (1)**
Chair: Douglas Hanahan, Lausanne

Gerhard M. Christofori, Basel
Regulatory circuits of EMT and metastasis

Mina J. Bissell, Berkeley
Why don't we get more cancer?
The crucial role of extracellular matrix and microenvironment in metastasis and dormancy

Joan Massagué, New York
Deconstructing metastasis

15:30 – 16:00 *Break*

16:00 – 17:00 **Mechanisms of Metastasis (2)**
Chair: Susan M. Gasser, Basel

Klaus Pantel, Hamburg
Circulating tumour cells: Biology and clinical relevance

Luis A. Diaz, Baltimore
Circulating tumour DNA

17:15 – 18:45 **Award ceremony**
Charles Rodolphe Brupbacher Prize for Cancer Research 2015
All participants are kindly invited to attend

18:45 – 19:30 *Apéro*

Friday, January 30, 2015

08:30 – 10:00 Breakthroughs in Cancer Therapy (1)
Chair: Roger Stupp, Zurich

Louis Staudt, Bethesda
New lymphoma therapies inspired by functional and structural genomics

Lewis C. Cantley, New York
Targeting phosphoinositide 3-kinase for cancer therapy

Francesco Lo-Coco, Rome
Curing acute promyelocytic leukemia without chemotherapy

10:00 – 10:30 Coffee break

10:30 – 11:30 Breakthroughs in Cancer Therapy (2)
Chair: Alexander Knuth, Qatar

Patrick Baeuerle, Munich
T cell-engaging bispecific antibody constructs for treatment of leukemia

Alexander M.M. Eggermont, Paris
(Re)Naissance of cancer immunotherapy: breaking tolerance

11:30 – 12:00 Young Investigator Awards
Referees: Lauri A. Aaltonen, Miriam Merad, Bernard W. Stewart

Awards presented by Mme. F. Brupbacher, President of the Foundation and Klaus W. Grätz, Dean of the Medical Faculty

Abstracts
Eingeladene Redner

Abstracts
Invited Speakers

Humanized mice for cancer research

Richard A. Flavell

Department of Immunobiology and Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, USA

Mice carrying a human hematopoietic and immune system represent a valuable tool to study human immunity, inflammation and hematopoiesis in *in vivo* settings. However, in currently available models of humanized mice, the development and/or function of many human immune cell types is suboptimal. This condition is mainly due to a reduced or absent cross-reactivity of molecules produced by the mouse host on the human cells. To circumvent this limitation, we generated several strains of mice, in which we replaced mouse genes by their human counterparts. These novel strains of recipient mice improve the engraftment, development and function of multiple lineages of human immune cells in the mouse. These models are useful to model and to study human hematopoiesis and immune responses in physiological as well as pathological conditions such as inflammation or neoplasia.

Gut microbiota-dependent antitumor efficacy of CTLA4 blockade

Marie Vétizou, Jon Pitt, Romain Daillere, Sophie Viaud and Laurence Zitvogel

Gustave Roussy Comprehensive Cancer Center, Villejuif-Grand-Paris, France

We showed that gut commensals are involved in the adjuvant effects of cyclophosphamide (Viaud et al 2013, Iida et al. 2013). The cytotoxic T lymphocyte antigen-4 (CTLA-4)-blocking antibody ipilimumab is a FDA and EMEA-approved treatment for metastatic melanoma despite frequent grade II-III colitis. To uncouple efficacy and toxicity, we analyzed the changes and role of gut microbiota during CTLA4 blockade. Here, we show that broad spectrum antibiotic cocktail ATBx, imipenem and colistine severely compromise the efficacy of CTLA4 blockade while vancomycine ameliorates it. ATBx reduced intratumoral leukocyte, and more specifically Tc1 accumulation, impaired the upregulation of inducible costimulator (ICOS) on T cells and compromised Foxp3 loss in CD4+Lag3+ cells, all being hallmarks of ipilimumab efficacy. A loss of CTLA4 blockade efficacy was also observed in germ free mice. Anti-CTLA4 Ab induced a loss of the epithelial barrier integrity, a dramatic upregulation of RORgt and IFNg in colonic LP T cells and increased Goblet cells numbers in the small and large intestine. Efficacy as well toxicity of CTLA4 blockade were worsened by anti-ICOS and anti-IL-10 Ab. Data on the biofilm pyrosequencing will be available to delineate which Gram negative bacteria plays a probiotic role in these synergistic effects. Our data confirm the importance of the gut microbiota in the clinical efficacy and potentially toxicity of CTLA4 blockade.

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Microbiota and response to cancer therapy

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Mammals live in partnership with a rich commensal microbiota on the body epithelial surfaces. This partnership is critical for tissue formation, metabolism and the development and function of the innate and adaptive resistance. The gut microbiota plays a role not only in local tissue formation and the development of mucosal immunity, but has also systemic effects regulating inflammation, immunity and metabolic functions. The microbiota is also closely linked to cancer development both locally, as is the case for colorectal carcinoma, and at distant sites as illustrated by mammary carcinoma, hepatocellular carcinoma and lymphoma. The role of inflammation in cancer is well documented and myeloid cells represent a major component of the tumor microenvironment where they play a dual role inducing anti-tumor immune responses but mostly promoting immune evasion and tumor progression. Therefore, strategies aiming at reprogramming the tumor microenvironment represent a promising immunotherapy approach. We have recently shown that microbiota perturbation impairs the response of subcutaneous cancers to CpG oligonucleotide-immunotherapy and platinum chemotherapy, and in both cases innate myeloid cells are responsible for the impaired response albeit through distinct mechanisms. Our data suggest that the composition of the commensal microbiota modulates the response to cancer immunotherapy and chemotherapy by regulating myeloid-derived cell function in the tumor microenvironment. Thus, providing new targets and possibilities for therapeutic intervention.

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Molecular mechanisms of colon cancer metastasis: toward clinical applications

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The direct cause of cancer death is often its metastasis to the vital organs. Metastasis is achieved through a multistep cascade of events, and therefore inefficient as a whole. Despite the efforts to find mutations that are responsible for metastasis, relatively few such genetic changes have been found, leading to the speculation that metastasis is driven by the mechanisms for physiological and/or pathological body reactions.

Recently, we have shown that *AES/Aes (Amino-terminal enhancer of split*) gene encodes colorectal cancer (CRC) metastasis suppressor Aes that functions as an endogenous inhibitor of Notch signaling that plays a variety of roles in cancer in a context-dependent manner. Expression of Aes is decreased in liver metastases compared with primary colon tumors in both mice and humans. Aes inhibits Notch signaling by converting active Rbpj transcription complexes into repression complexes on insoluble nuclear matrix. In tumor cells, Notch signaling is triggered by ligands on adjoining blood vessels, and stimulated transendothelial migration. Upon introduction of homozygous *Aes* knockout mutation into adenomatous epithelium of $Apc^{+/\Delta 716}$ intestinal polyposis mice, their tumors become malignant due to Notch signaling activation, showing submucosal invasion and intravasation. Consistently, transendothelial migration is increased significantly, when CRC cells are activated for Notch signaling and placed on an endothelial cell layer in culture. Thus, reduced level of Aes and stimulation of Notch signaling are implicated in the invasion and intravasation of CRC cells during metastasis.
Although Notch signaling is involved in cell migration during the nervous system development, the precise molecular mechanisms remain to be elucidated. Now, we have investigated how Notch signaling stimulates CRC metastasis. Here we show that one of the genes induced by Notch signaling in CRC is *DAB1/Dab1*. Genetic depletion of Dab1 suppresses cancer invasion and metastasis in the Notch signaling-activated mice. Dab1 is phosphorylated by Abl tyrosine kinase, and phosphorylated Dab1 activates Abl reciprocally. Consistently, inhibition of Abl suppresses cancer invasion in mice. Furthermore, we show that one of the targets of Abl Tyr-kinase is Rac/RhoGEF protein Trio, and that phosphorylation at its Tyr residue 2681 (pY2681) causes Rho activation in CRC cells. The unphosphorylatable Tyr-to-Phe mutation Trio(Y2681F) reduces the RhoGEF activity, and inhibits invasion of CRC cells. Importantly, Trio(pY2681) correlates with significantly poorer prognosis of CRC patients after surgery.
These results indicate that Trio(pY2681) is one of the downstream effects by Notch signaling activation in CRC, and can be a prognostic marker, helping determine the therapeutic modality of CRC patients.

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Therapeutic targeting of the tumor microenvironment

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Amidst an era of interrogating with increasing accuracy the aberrations in the genomes and epigenomes of cancer cells that endow them with malignant potential is another dimension to the phenotypic complexity of human cancer – the tumor microenvironment (1). It can now be appreciated that virtually every form of human cancer depends to some degree on recruited and corrupted accessory cells to facilitate their development, progression, and resilience, particularly in the face of therapy. A roster of ostensibly normal cells collaborate with overt cancer cells to establish tumor microenvironments, and by so doing contribute to the acquisition of necessary capabilities (2), enabling the 'hallmarks of cancer' (1). Given the adaptability of many forms of human cancer to hallmark-targeting drugs aimed at the mutation-prone cancer cells, it is arguable that the future of effective cancer therapy will involve multi-pronged attacks that include the stromal cells of the tumor microenvironment (and their effector mechanisms) as therapeutic targets (3).
The Hanahan laboratory studies mechanisms of tumor development and progression in genetically engineered mouse models of human cancer, including in particular the contributions of the tumor microenvironment. Current results on functions and therapeutic targeting of cell types and functions embodied in the tumor microenvironment will be presented.

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Targeting the host cells in B cell lymphoma - Is it superior to chemotherapy?

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Indolent B cell lymphomas are malignancies, which are derived from mature B cells and characterized by a slow tumour progression with subsequent bone marrow failure. In spite of an enormous improvement of anti-lymphoma therapies in recent years, allogeneic stem cell transplantation remains the only way of cure, but is associated with significant treatment associated morbidity and therefore not applicable to most patients.

An increasing amount of data supports the finding that the tumour microenvironment actively contributes to disease progression by fostering cell proliferation and survival of malignant cells (1). Our work is focused on understanding how bone marrow derived stromal cells (BMSCs) contribute to cell survival and drug resistance of malignant B cells, as residual lymphoma cells in the bone marrow inevitably remain a pool of cells that cause disease relapse after an apparent clinical success with immunochemotherapies.

Notably, the cell-cell communication between BMSCs and malignant B cells is bi-directional and based on the activation of signalling pathways in both cell compartments. In contrast to normal peripheral blood B cells, malignant B cells reprogram BMSCs, which then become reminiscent of cancer-associated fibroblasts (CAFs), based on morphological changes and alterations in gene expression. Similar to CAFs in solid tumours, activation of NF-κB in BMSCs contributes to a pro-inflammatory environment, which is essential for the survival of malignant cells (2). We identified activation of protein-kinase C as a key upstream regulator of NF-κB in stromal cells in chronic lymphocytic leukaemia and mantle cell lymphoma (3). Since the activation of this pathway is an essential pre-requisite for disease progression *in vivo*, targeting up-stream regulators of stromal NF-κB may constitute an alternative therapeutic approach to classical cytotoxic therapies.

The activation of the PKC- NF-κB pathway requires a direct cell-cell contact and is activated in various different mesenchymal cells, indicative of a conserved induction-signal: A growing amount of evidence suggests that the NF-κB pathway collaborates with Notch signalling. Our data further indicate that Notch activation in BMSCs is another important regulator of microenvironment-mediated protection of malignant B cells.

A major hurdle for the development of successful therapies remains the inherent genomic instability of tumour cells, based on secondary mutations and the outgrowth of tumour subclones selected for drug resistance. Therefore it is tempting to speculate that pharmacological interference with the activation of the microenvironment is more likely to stop disease progression and to eradicate lymphoma cells, because -until further evidence- stromal cells are not driven by clonal evolution.

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Seeing cancer in context: imaging reveals contributions of the microenvironment to therapy resistance and metastasis

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Solid tumors are aberrant tissues. Like organs, solid tumors are composed of cancer cells and stroma. The stroma is the supportive framework of the organs and includes the extracellular matrix, fibroblasts, adipocytes, cells of the vascular system, and immune cells. Interactions between epithelium and stroma are essential for normal organ development and for tumor progression. In solid tumors, the stroma is also known as the tumor microenvironment.

We study how the tumor microenvironment influences cancer cells in the context of drug resistance and metastasis. We use mouse models of breast and pancreatic cancer together with real-time spinning disk microscopy in living mice (intravital imaging). This allows us to study how cancer cell proliferation, migration, and survival are influenced by stromal components in real-time.

Breast cancer recurs in 20% of patients within 10 years after apparently successful treatment with chemotherapy. When resistance to chemotherapy develops, few effective treatment options are available. Surprisingly little is known about how cancer cells in intact tumors develop resistance to classical chemotherapy. Most knowledge on the responses has been obtained from cell culture or xenograft animal experiments, but such experiments are not always predictive of drug responses in patients. We previously used intravital imaging to show that monocytes are recruited to tumors after chemotherapeutic treatment - after cancer cell death was observed. We determined that these monocytes, recruited to tumors through activation of the chemokine receptor CCR2, contributed to chemoresistance, as chemotherapy lead to greater tumor shrinkage in tumors of host mice lacking CCR2.

The prognosis of metastatic breast cancer is poor. Traditionally, studies on metastasis have relied primarily on measurements made at the end-point of the process, the establishment of micro- or macro-metastases. However, the metastatic process is dynamic and characterized by the ability of cancer cells to move from one part of the body to another: cells exit the primary tumor, invade the local tissue, enter blood or lymphatic vessels, and are transported to a distant site where they exit the vessels and move into the tissue. Thus, a different level of understanding of metastasis might be achieved using technologies that can follow these dynamic processes *in vivo* and in real-time.

We are using intravital imaging to determine how interactions between cancer cells and stromal cells influence metastasis. We have identified significant differences in the types and amount of chemokines that are secreted by the cancer cells in metastatic and non-metastatic tumors. Strikingly, tumors grow slower and metastasis is greatly reduced in mice that lack the receptor for one of the chemokines that specifically is secreted by metastatic cancer cells. Ongoing studies using intravital imaging during the formation of lung and liver metastasis are addressing how signaling between cancer cells and stromal cells regulates invasion and promotes metastasis.

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Personalisierte Krebsmedizin

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Die Inzidenz von Krebserkrankungen steigt stetig. Im Vergleich zum Jahr 2008, in welchem weltweit 12.7 Mio. neue Fälle und 7.6 Mio. Todesfälle registriert wurden, waren es im Jahr 2012 bereits 14.2 Mio. beziehungsweise 8.2 Mio. Für das Jahr 2025 sagen die Statistiker beinahe 20 Mio. neue Fälle voraus. Der Hauptgrund dieses Anstiegs ist der steigende Altersdurchschnitt der Bevölkerung. Was kann man unternehmen, um diese Zunahme zu stoppen?
In erster Linie müssen wir die Bevölkerung an die Wichtigkeit von regelmässigen ärztlichen Kontrollen erinnern, denn einige Krebsarten, z.B. Dickdarmkrebs, können vorgebeugt werden, und andere können aufgrund einer frühzeitigen Diagnose geheilt werden. Darüberhinaus sind wir heute in der Lage, mit Hilfe modernster Technologien, Unterschiede zwischen den genetischen Profilen der Tumorzellen und denen des gesunden Gewebes des Patienten zu identifizieren. Diese Experimente haben gezeigt, dass Tumore die pathologisch gleich klassifiziert wurden, genetisch sehr unterschiedlich sein können. Überraschenderweise bestehen aber auch zwischen den gesunden Geweben verschiedener Patienten wesentliche Unterschiede. Das Ziel der „personalisierten Krebsmedizin" ist es, herauszufinden welche der zahlreichen Unterschiede entscheidend für den Verlauf der Erkrankung (Prognose) ebenso wie für den Erfolg der Behandlung sind, sei es Chemo-, Radio- oder Immuntherapie, sowie auch für die Nebenwirkungen der jeweiligen Therapieform. In Zukunft sollte jeder Patient „massgeschneidert", mit dem therapeutischen Ansatz der seinen Tumor am effizientesten und mit minimalsten Nebenwirkungen bekämpft, behandelt werden. Wie realistisch ist diese Vision, und wo stehen wir heute?

Mutational processes in human cancer

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All cancers are caused by somatic mutations. However, the processes underlying the genesis of somatic mutations in human cancer are remarkably poorly understood. Recent large-scale cancer genome sequencing initiatives have provided us with new insights into these mutational processes through the mutational signatures they leave on the cancer genome. In this talk I will review the mutational signatures found across cancer and consider the underlying mutational processes that have been operative.

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The Cancer Epigenome

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Epigenetic processes are reinforced by interactions between covalent chromatin marks such as DNA methylation, histone modifications and variants. These marks ultimately specify the locations of nucleosomes particularly with respect to transcriptional start sites and other regulatory regions. We have developed a new methodology to simultaneously map nucleosomal positioning and DNA methylation on individual molecules of DNA and show that the methylation of CpG islands at the transcriptional start sites of key tumor suppressor genes results in the stable placement of nucleosomes at the transcription start site. Inhibition of DNA methylation by 5-azanucleoside treatment results in an immediate inhibition of DNA methylation and a sequence of downstream events ultimately resulting in the eviction of the nucleosomes from the transcription start site and the activation of gene expression.

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Molecular Genetics 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 (GCB-DLBCL) or from post-GC B cells resembling *in vitro* activated B cells (ABC-DLBCL), suggesting that these two diseases utilize distinct oncogenic pathways. Accordingly, 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 mutant genes regulating important pathways involved in GC development, including those involved implicated in chromatin remodeling, transcriptional control of apoptosis and differentiation, NF-kB activation and immune escape. Recent results have also identified a pervasive mutational deregulation of a transcription factor network (MYC-MEF2B-BCL6-FOXO1) involved in the control of GC development. The normal role of these transcription factors and the consequence of their mutations in DLBCL have been investigated using GC-directed conditional transgenic mice.

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Mouse models for lung cancer: Tumor heterogeneity and cell-of-origin of thoracic tumors

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Lung cancer and mesotheliomas are amongst the most lethal human malignancies with poor prognosis. The majority of these tumors are associated with carcinogen exposure; specifically smoking and asbestos. Small cell lung cancer (SCLC) and mesothelioma patients show very poor survival statistics due to their late detection, invasive and high metastatic potential, and chemo-resistance. Using the Rbf/f;p53f/f mouse model for SCLC, we found that the tumors are often composed of phenotypically different cells, characterized by mesenchymal and neuroendocrine markers. These cells often share a common origin. Crosstalk between these cells can endow the neuroendocrine component with metastatic capacity, illustrating the potential relevance of tumor cell heterogeneity in dictating functional tumor properties. Also specific genetic lesions appear to be associated with metastatic potential. We have studied the nature of this crosstalk and identified the components responsible for paracrine signaling and the downstream effector pathway critical for promoting metastatic spread.
We have also evaluated the relevance of additional lesions that were frequently acquired in the mouse SCLC, such as amplification of Myc and Nfib. Therefore, we have derived ES cells from Rbf/f;p53f/f, equipped these cells with an exchange cassette in the ColA1 locus, and shuttled a conditional L-Myc and Nfib under a strong promoter into this locus. This accelerated tumorigenesis and resulted also in a shift in the metastatic phenotype.
To investigate the cell-of-origin of thoracic tumors, we have inactivated a number of tumor suppressor/oncogene combinations (Trp53, Rb1, Nf2, Cdkn2ab-p19Arf, mutant Kras) in distinct cell types by targeting Cre-recombinase expression specifically to Clara cells, to neuroendocrine cells, alveolar type II cells and cells of the mesothelial lining (origin of malignant mesothelioma) using adenoviral or lentiviral vectors with Cre recombinase driven from specific promoters. Dependent on the induced lesions and the cell-type specific targeting, SCLC, non-SCLC, or mesothelioma could be induced. We show that multiple cell types can give rise to these tumors but that the cell-of-origin is an important factor in determining tumor phenotype.
Our data indicate that both cell type specific features and the nature of the oncogenic lesion(s) are critical factors in determining the tumor initiating capacity of lung (progenitor) cells. Furthermore, the cell-of-origin appears to influence the malignant properties of the resulting tumors.

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Epithelial Stem Cells in Silence, Action and Cancer

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Stem cells have the ability to self-renew long term and differentiate into one or more tissues. Typically, stem cells are used sparingly to replenish cells during normal homeostasis. However, even stem cells that are quiescent must be able to respond quickly to injury in order to fuel rapid tissue regeneration. How stem cells balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair. Increasing evidence suggests that the regulatory circuitry governing this balancing act is at the root of some types of tumors, both in mice and in humans.

The skin is an excellent model system to understand how stem cells function in normal tissue generation and how this process goes awry in cancer. We've identified and characterized at a molecular level the stem cell niches within the epidermis, hair follicle, and glands of the skin. We've learned that during normal homeostasis, stem cell behavior is controlled not only through cues received from their microenvironment, but also through signals emanating from their differentiating lineages. We've been dissecting how extrinsic niche cues, particularly those eliciting changes in BMP, WNT and SHH signaling, trigger a cascade of chromatin-associated and transcriptional changes within stem cells that governs their activation during tissue development, homeostasis and hair regeneration. We've applied this knowledge in exploring how stem cells change as they exit their niche and embark upon a specific lineage program or alternatively participate in wound-repair following injury. Our findings have provided new insights into our understanding of the normal process of stem cell activation during homeostasis and wound-repair, and in so doing, we began to realize that malignant progression hijacks these basic mechanisms which are essential for all tissues. We've now focused on how stem cell behavior goes awry during tumor progression. We've purified and characterized functional skin tumor-initiating cells at near homogeneity. We also developed a new method to knockdown genes specifically in skin and oral progenitors, enabling us to screen not only the differences between these cancerous and normal stem cells, but also the myriad of gene alterations surfacing from the Human Cancer Sequencing project. We've also carried out whole genome-wide RNAi screens for oncogenic growth of stratified epithelia. Our screens have illuminated new oncogenes and tumor suppressors for squamous cell carcinomas, among the most prevalent and life-threating cancers world-wide that include cancers of lung, esophagus, breast, cervix, prostate, throat and oral tissues. Our findings are unearthing new targets for cancer therapeutics, as well as for delving deeper into understanding the mechanisms that underlie malignancy and metastasis.

Wnt / beta-catenin and cooperative signaling sytems in cancer stem cells

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Combined β-catenin gain-of-function and Bmp receptor loss-of-function mutations in mice by using K14-cre resulted in the rapid growth of aggressive squamous cell carcinoma (SCC) in the salivary glands. High Wnt/β-catenin expression and low Bmp signaling also characterize human salivary gland SCC and human head and neck SCC in general. By fluorescence-activated cell sorting, we identified a distinct population of transplantable and hyper-proliferative cancer stem cells. When injected into the back skin of NOD/SCID mice, as few as 500 enriched-cancer stem cells produced fast-growing tumors. Cells could be propagated as sphere cultures and formed lose, undifferentiated aggregates. Chemical targeting of Wnt signaling by small molecule β-catenin inhibitors and siRNAs resulted in differentiated glandular structures that resembled salivary gland acini and this treatment inhibited tumor growth in mice.

The cancer stem cells expressed a gene signature that was associated with pluripotency (Nr5a2, Hells, Dppa5 and others), and several histone-modifying genes were up-regulated (Ash2, MLL, Rnf2 and others). Moreover, a nuclear complex of β-catenin and MLL promoted the self-renewal and H3K4 tri-methylation in the cancer stem cells. Blocking β-catenin-MLL interaction and siRNAs against β-catenin or MLL abrogated proliferation and H3K4 tri-methylation, induced differentiation of the spheres into acini-like structures, and also reduced tumor growth. Remarkably, these treatments resulted in the translocation of β-catenin to the cytoplasm, and MLL was degraded. Taken together, these findings suggest that we have uncovered new nuclear mechanisms by which Wnt/β-catenin signals remodel chromatin and control the induction and maintenance of cancer stem cells.

Compound mutant mice that carried gain-of-function β-catenin and HGF mutations (the Met ligand) under the WAP promoter (which targets epithelia

of mammary glands post-partum) were also produced. Wnt-Met post-partum mice developed rapid and aggressive mammary gland tumors, which were basal and triple-negative. In tumor cells were treated with β-catenin or Met inhibitors, gene expression profiling revealed genes controlled by Wnt signaling which are essential for self-renewal (Birc5, Top2a, Hells, Aurkb and others), while also indicating Met signaling regulated genes important for epithelial differentiation (CXCR4, Krt14, Vegf, Gata3 and others). We also used mouse genetics to conditionally ablate the CXCR4 gene in Wnt-Met mice resulting in significantly delayed growth of tumours which were triple mutants. When Wnt-Met enriched-cancer stem cells were transplanted into cleared mammary gland fat pads of NOD/SCID/IlR2-/- mice, one hundred cells generated tumor outgrowths four weeks later. Moreover, growth of mammospheres treated with β-catenin inhibitors or siRNAs was significantly reduced, indicating that Wnt signaling controls self-renewal. In contrast, inhibition of Met or CXCR4, or siRNAs blocked differentiation into hollow alveoli.

We performed therapy experiments in the Wnt-Met mouse model. Animals treated with β-catenin or Met inhibitors showed a moderately decreased growth of tumors; however, strong inhibition was found after triple treatment using CXCR4, Met and β-catenin inhibitors. Furthermore, the expression of a mouse Wnt-Met 322 gene signature was predictive of poor survival of human patients diagnosed with ER-negative breast cancers. Taken together: Wnt-Met activation in mouse mammary glands rapidly produced aggressive basal/triple-negative tumors and expanded cancer stem cells, in which the two signaling systems control different functions. Combination therapies targeting Wnt, Met and CXCR4 significantly delayed tumor development.

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Normal and Neoplastic Stem Cells

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Following embryonic development, most of our tissues and organs are continuously regenerated from tissue/organ specific stem cells. The principal property that distinguishes such stem cells from their daughter cells is self-renewal; when stem cells divide they give rise to stem cells (by self-renewal) and progenitors (by differentiation). In most tissues only the primitive stem cells self-renew. Stem cell isolation and transplantation is the basis for regenerative medicine. In the 1980-90s, we isolated mouse and human hematopoietic (blood-forming) stem cells (HSCs), and found that their method of isolation depleted all detectable breast cancer cells from mobilized peripheral blood (MPB). Patients' MPB or cancer-free HSCs were used in autologous transplantations to rescue the hematopoietic system of women who underwent high dose combination chemotherapy for stage IV breast cancer. The results with MPB autologous transplantation were similar to those reported previously, with a 12–15-year overall survival rate of ~7%. In contrast, for contemporaneous patients in a small phase I/II trial at Stanford who received autologous transplants with cancer-free HSCs, this survival rate was ~33%. By chi-square analysis, MPB vs. cancer-free HSC transplantations were significantly different in terms of both progression-free and overall survival at all time points tested from 20 months to 12-15 years after transplantation.

In considering stem cells and cancer, self-renewal is dangerous and therefore strictly regulated. Poorly regulated self-renewal can lead to the genesis of cancer stem cells, the only self-renewing cells in the cancerous tumor. The Weissman lab has followed the progression from HSCs to myelogenous leukemias. We found that the developing cancer clones progress at the stage of HSCs, until they become fully malignant. At this point, the "leukemia" stem cell moves to a stage of a downstream oligolineage or multilineage progenitor that has evaded programmed cell death and programmed cell removal, while acquiring or keeping self-renewal. In the case of chronic myelogenous leukemia, bcr-abl^{+} HSC clones outcompete normal HSCs in the chronic phase. The transition from the chronic phase to myeloid blast crisis results in the leukemia stem cells appearing in the granulocyte-macrophage progenitor (GMP) stage, and is accompanied by cell intrinsic activation of β-catenin, inhibitable by transfection with axin, which holds β-catenin and the enzyme glycogen synthase kinase–3β [GSK3β] in a complex, allowing GSK3β to phosphorylate β-catenin. This marks it for proteolytic degradation. In 4/7 patients studied, this resulted from stage-specific (GMP) mis-splicing

of the GSK3β message, deleting the kinase domain, and allowing β-catenin to translocate to the nucleus where it co-transcribes a family of genes involved in proliferation, including c-myc. As this mis-splicing only occurred in the GMP stage leukemia stem cells (LSC), identification and purification of all stages of hematopoiesis from HSCs was necessary to unravel the molecular mechanisms involved in CML induction (at the HSC stage) and progression to blast crisis at the GMP stage. To date, although the general method to identify and isolate normal stem and progenitors has been available since our studies in the 1980s, the method has hardly been used to elucidate stem and progenitors in other tissues, and therefore to be able to isolate incipient and actual cancer stem cells derived from these tissues.

Having isolated HSC and MPPs and in AML their incipient and LSC counterparts, we used comparative gene expression analyses to reveal genes up or down regulated in LSCs of both mouse and human AMLs. One of the upregulated genes was CD47, shown by Oldenberg to be an age marker on mouse red cells by acting as a 'don't eat me' signal for splenic and bone marrow type macrophages. Years ago Lagasse and Weissman showed that neutrophiles overexpressing the anti-apoptotic protein bcl2 did not undergo programmed cell death (PCD), yet at the precise time when wild-type neutrophiles showed the internal changes of apoptosis, like those neutrophiles expressed 'eat me' signals for macrophages, a process we call programmed cell removal (PrCR). While there are many ways to defeat PCD and senescence, there appears to be one dominant method to avoid PrCR—the expression of the cell surface "don't eat me" protein CD47, the ligand for macrophage SIRPα. Over the past 4 years we have found that all primary cancers tested express CD47 to overcome expression of "eat me" signals such as calreticulin and asialogylycoproteins. We obtained and also made antibodies that block the CD47–SIRPα interaction, and showed that these antibodies enable phagocytosis and killing of the tumor cells *in vitro* and *in vivo*. All tested human patient solid tumors and lymphomas/leukemias/myelomas grown in immune deficient mice following orthotopic transplantation are susceptible to phagocytosis in the presence of anti-CD47 blocking antibodies, including a humanized antibody of the IgG4 isotype. Our anti-CD47 antibodies synergize with other IGG1 human anti-cancer antibodies to limit or eliminate the growth and metastasis of primary human cancer xenografts, e.g., with Rituximab for non-Hodgkin lymphoma, Cetuximab for EGF-R+ cancers such as colorectal cancers, and Trastuzimab for Her2+ breast cancers. In addition, we have prepared a second class of anti-CD47 reagents by modifying the CD47 binding domain of Sirpa, either as a monomer or as an IgG4 fusion protein. Both the monomer and IgG4 fusion proteins also synergize with other anti-cancer IgG1 antibodies. The humanized IgG4 anti-CD47 antibody Hu5F9G4 is currently in phase 1 clinical trials. Finally anti CD47 treated DLD1-ova human colorectal cancer cells leads to not only phagocytosis, but also macrophage cross-presentation of the ovalbumen peptides to ova-specific mouse CD 8 T cells. Vaccination of mice with these antigen-presenting macrophages lead to effective immunity to subsequently transferred syngeneic mouse leukemia cells transfected with the ova gene. Thus, anti-CD47 MAbs cancer therapies may include both innate and adaptive immunity in cancer patients, a possibility we will test in the patients in the clinical trials; and, of course, justify the approaches to normal and neoplastic stem cell biology that has led to these trials.

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Regulatory circuits of EMT and metastasis

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Cancer cell invasion into the surrounding tissue is a critical hallmark of cancer metastasis. Ninety per cent of all cancers originate from epithelial tissues and, to leave the primary tumor and invade their environment, tumor cells dissolve their cell-cell contacts and adjust their cell-matrix adhesion sites to a more transient, migratory and invasive mode. Such temporary and reversible phenomenon is known as epithelial-to-mesenchymal transition (EMT). The distinct stages of an EMT are tightly regulated by various signaling pathways and transcriptional control circuits and involve activation and repression of a large number of genes that modulate the invasive behavior of cells (1). For example, Sox4 is indispensable for EMT and cell survival *in vitro* and for primary tumor growth and metastasis *in vivo*. Sox4 directly regulates the expression of *Ezh2*, encoding the Polycomb group histone methyltransferase that trimethylates histone 3 lysine 27 (H3K27me3) for gene repression, and thus has an impact on the epigenetic regulation of gene expression during EMT (2).

Klf4 is another example of a transcription factor playing a critical role in EMT. Loss and gain of function experiments demonstrate that the down-regulation of Klf4 expression is required for the induction of EMT *in vitro* and for metastasis *in vivo*. Reduced Klf4 expression is correlated with shorter disease-free survival of subsets of breast cancer patients. Klf4 acts as a transcriptional activator of epithelial genes and as a repressor of mesenchymal genes. Specifically, increased expression of Jnk1 (*Mapk8*) consequent upon down-regulation of its transcriptional repressor Klf4 is required for EMT and cell migration (3).

On the other hand, the levels of Tead2, the DNA binding effector of the Hippo-Warts signaling pathway, increase in the nucleus of cells during an EMT, thereby directing a predominant nuclear localization of relevant co-factors Yap and Taz. Genome-wide chromatin immunoprecipitation/next generation sequencing in combination with gene expression profiling reveals the transcriptional targets of Tead2 during EMT. Among these, zyxin contributes to the migratory and invasive phenotype evoked by Tead2 (4).

Many of the signaling pathways and transcription factors critical for the regulation of EMT are well known for their functions in the homeostasis of embryonic and somatic stem cells. We find that cells undergoing an EMT exhibit several hallmarks of stem cells and are more tumorigenic, as compared to their epithelial counterparts. Notably, the expression of several angiogenic factors is upregulated during an EMT, and the increased expression of VEGF-A is required for the tumorigenicity of invasive mesenchymal cells. We conclude that a high angiogenic potential is an intrinsic feature of tumor-initiating cells/cancer stem cells (5).

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Why don't we get more cancer? The crucial role of extracellular matrix and microenvironment in metastasis and dormancy

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The work from our laboratory in the last three decades has provided much impetus for the current recognition and acceptance of the importance of context/microenvironment and extracellular matrix (ECM) in regulation of gene expression, and has underscored the plasticity of both the differentiated state and tumors. I will discuss why and how we developed, and use, 3-dimensional models of normal mammary gland and mammary tumors from both mice and humans to understand breast cancer, and will present recent work, shedding light on why tissue and organ architecture should become also a parameter in cancer research, and how architecture can regulate tissue-specificity as well as the plasticity of tumors. I will also discuss newer and more complex models we have developed to understand metastasis and dormancy and a screen that has allowed us to discover a new class of 'oncogenes' in the EGFR/PI3 Kinase.

We have shown a mechanism to underscore the model of dynamic reciprocity and how the ECM and basement membrane signal to nucleus, via intricate interactions with nuclear actin to provide cell and tissue quiescence, and discovery of a novel movement through kinetic imaging of how a unit of tissue function in the mammary gland (an acinus) is formed in the normal breast, lost in malignancy and reformed by controlling the microenvironment and restoring tissue context and architecture.
We suggest these concepts and models have profound implications for diagnosis, prognosis, drug resistance, dormancy and therapy of cancer.

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Deconstructing metastasis

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Identifying the mechanisms that turn disseminated cancer cells into metastasis initiating entities is critical for a better understanding and treatment of cancer. Most cancer cells that leave a tumor die, raising questions about what kills these cells and what sometimes allows their survival and tumor re-initiation. By means of cell sorting *in vivo*, we developed mouse models of metastasis by different tumor types to different organ sites. Using these models and human tumor samples we identified genes and pathways that enhance cancer cell infiltration, survival, and eventual colonization of the bone marrow, the lungs or the brain. The encoded proteins and microRNAs prime tumor cells for metastasis in animal models and predict relapse in patients.
In mammary, lung, renal, and colorectal carcinomas the mediators of disseminated cancer cell survival and fitness fall into two general classes. One class includes factors that amplify survival (PI3K/AKT) or fitness pathways (Notch, Wnt). The other includes factors that form paracrine loops with stromal macrophages, myeloid precursors, and fibroblasts to benefit the metastatic tumor. The diversity of metastatic mechanisms emerging from this work illuminates the basis for organ-specific metastasis and also the challenge of therapeutically targeting this process.
Prompted by these findings, we focused on brain metastasis from breast and lung cancers. We reasoned that the unique nature of the brain parenchyma and its stringent barriers against infiltration by circulating cancer cells might select for common mediators of metastasis in different cancers. Brain metastases occur in 20-40% of advanced stage cancers and represent the most prevalent intracranial malignancy in adults. We found that circulating cancer cells that infiltrate the brain die massively from exposure to astrocytes. Reactive astrocytes generate plasmin, which mobilizes the pro-apoptotic cytokine FasL to kill infiltrating cancer cells. Plasmin additionally cleaves a key cell adhesion protein on cancer cells, L1CAM. However, brain metastatic cells can evade this attack by expressing serpin inhibitors of plasminogen activator.
The surviving cancer cells adhere to, and stretch over brain capillaries by means of L1CAM. This step is required for the initiation of metastatic outgrowth. Metastatic cells proliferate on the abluminal basal lamina of the capillaries, generating a sheath that engulfs the local capillary network and eventually forms a macrometastatic tumor. When devoid of L1CAM, circu-

lating cancer cells can still infiltrate tissue and survive in the short term, but they fail to coopt the vasculature and to expand. In mouse models, we find that L1CAM is required for metastatic outgrowth not only in the brain but also in bones and lungs. L1CAM-mediated vascular cooption may constitute a common requirement for metastatic colonization by multiple tumor types in different organs sites. Our findings point at a singular opportunity to combat metastasis.

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Circulating tumour cells: Biology and clinical relevance

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Sensitive methods have been developed to capture circulating tumor cells (CTCs) in the peripheral blood at the single cell level in cancer patients (Pantel et al., Nature Rev Cancer 2008; Kang & Pantel, Cancer Cell 2013). CTCs are usually detected by immunostaining or RT-PCR assays, and more recently by the EPISPOT assay which measures the number of cells releasing/secreting tumor-associated marker proteins. Interestingly, detection of cell-free nucleic acids released by tumor cells such as tumor-associated DNA or microRNAs into the blood might become an indirect way to detect micrometastatic disease (Schwarzenbach/Pantel et al, Nature Rev Cancer 2011 & Nature Rev. Clin. Oncol. 2014). At present, most CTC assays rely on epithelial markers and miss CTCs undergoing an epithelial-mesenchymal transition (EMT). New markers such as the actin bundling protein plastin-3 (Yokobori et al., Cancer Res. 2013) are not downregulated during EMT and not expressed in normal blood cells might overcome this important limitation and, therefore, increase the sensitivity of CTC assays. Recently, *in vivo* capture of CTCs with an antibody-coated wire placed into the peripheral arm vein has become feasible and allows now the capture for CTCs from approx. 1.5 liters of blood within 30 minutes. CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy (Alix-Panabieres & Pantel, Clin. Chem. 2013; Pantel & Alix-Panabieres, Cancer Res., 2013). Moreover, monitoring of CTCs before, during and after systemic therapy, which may include chemotherapy, hormonal therapy, and/or antibody therapy, might provide unique information for the future clinical management of the individual cancer patient and might serve as surrogate marker for response to therapy. Besides CTCs, the analysis of ctDNA and circulating microRNAs may provide complementary information as "liquid biopsy" (Pantel & Alix-Panabieres, Cancer Res., 2013; Pantel et al., Nature Med. 2013; Heitzer et al., Genome Med. 2013; Schwarzenbach et al., Nature Rev. Clini. Oncol., 2014). This information can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells (Wan, Pantel, Kang, Nature Med. 2013).

Circulating Tumour DNA

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Genotyping tumor tissue in search of somatic genetic alterations for actionable information has become routine practice in clinical oncology. Although these sequence alterations are highly informative, sampling tumor tissue has significant inherent limitations. A tumor tissue sample is a single snapshot in time, is subject to selection bias resulting from tumor heterogeneity, and can be difficult to obtain. Cell-free fragments of DNA are shed into the bloodstream by cells undergoing apoptosis or necrosis, and the load of circulating cell-free DNA (cfDNA) correlates with tumor staging and prognosis. Moreover, recent advances in the sensitivity and accuracy of DNA analysis have allowed for genotyping of cfDNA for somatic genomic alterations found in tumors. The ability to detect and quantify tumor mutations has proven effective in tracking tumor dynamics in real time as well as serving as a liquid biopsy that can be used for a variety of clinical and investigational applications not previously possible.

New Lymphoma Therapies Inspired by Functional and Structural Genomics

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Discovery of essential pathways that sustain the malignant phenotype can be greatly accelerated by joint application of functional and structural genomics. Genome-wide loss-of-function genetic screens based on RNA interference (RNAi) can reveal genes that are essential for cancer cell proliferation and survival. In parallel, high throughput resequencing of cancer genomics can uncover recurrent genetic aberrations that explain the dependence of cancer cells on particular signaling and regulatory pathways. Our laboratory focuses on diffuse large B cell lymphoma (DLBCL), a heterogeneous diagnostic category that is comprised of two prominent molecular subtypes, termed activated B cell-like (ABC) and germinal center B cell-like (GCB) (reviewed in ref. 1). These DLBCL subtypes are now viewed as molecularly distinct diseases since they arise from distinct stages of normal B cell development, require distinct recurrent genetic abnormalities to become malignant, have distinct cure rates with current chemotherapy regimens, and respond differentially to targeting agents.

The ABC DLBCL subtype has constitutive activation of the NF-κB pathway, which we traced by RNAi screening to the signaling adapter CARD11[2]. In approximately10% of ABC DLBCL tumor biopsies, we discovered recurrent CARD11 mutations that spontaneously activate NF-κB signaling. We also defined a "chronic active" form of B cell receptor (BCR) signaling that activates NF-κB in ABC DLBCLs with wild type CARD11[3]. Such ABC DLBCLs are killed by knockdown of BCR signaling components, such as the kinase BTK or components of the BCR itself. Over one fifth of ABC DLBCLs have mutations affecting the CD79B or CD79A subunits of the BCR that augment BCR signaling. In addition, NF-κB can be engaged in ABC DLBCL by MYD88, a key adapter in Toll receptor signaling[4], which sustains activating point mutations in 39% of these tumors.

To attack chronic active BCR signaling therapeutically, we initiated clinical trials in relapsed/refractory DLBCL of ibrutinib, an irreversible and highly selective inhibitor of BTK. Ibrutinib monotherapy induced a high rate of complete and partial responses in ABC DLBCL, while GCB DLBCL tumors rarely responded. Responses have been observed in "primary refractory" tumors that had never responded to any prior therapy and several patients have remained in complete remission for 1-4 years. Mutations in the BCR subunits CD79A and CD79B enriched for ibrutinib responders, but most responses

were observed in tumors with wild type BCR subunits, leading us to define non-genetic mechanisms that activate BCR signaling ABC DLBCL tumors. Tumors with MYD88 but not CD79A/B mutations never responded to ibrutinib, suggesting a genetic pathway to ABC DLBCL that is MYD88-dependent but BCR-independent.

While these responses to ibrutinib are encouraging, this agent will need to be paired rationally with other active drugs in lymphoma to achieve a high rate of cure in ABC DLBCL. Indeed, ibrutinib synergizes with inhibitors of the PI(3) kinase pathway, inhibitors of anti-apoptotic BCL2 family members, and various conventional chemotherapy agents[5]. Given its excellent safety profile and selective mechanism of action, we are hopeful that ibrutinib can be combined with both chemotherapy and other signaling modulators to achieve cures for these patients.

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Targeting Phosphoinositide 3-Kinase for Cancer Therapy

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Phosphoinositide 3-kinase (PI3K) is a central enzyme in a signaling pathway that mediates cellular responses to insulin and other growth factors. The signaling pathway, involving insulin, PI3K and the downstream components of AKT and mTOR, is highly conserved from worms and flies to humans and genetic analysis of the pathway has revealed a conserved role in this pathway regulating glucose metabolism and cell growth. Germline mutational events that lead to hyperactivation of the PI3K pathway result in hamartoma syndromes and cancers. Sporadic activating mutations in PIK3CA, encoding the p110alpha catalytic subunit of PI3K or inactivating mutations in PTEN, a phosphoinositide 3-phosphatases that reverses the effects of PI3K, are among the most common events evident in solid tumors. More than thirty drugs that target PI3K and other components of this pathway are in clinical trials for a variety of cancers. It is likely that PI3K pathway inhibitors will need to be combined with other drugs to be broadly effective. We have employed genetically engineered mouse models that develop cancers due to mutations in genes in the PI3K pathway and are using these models to explore the efficacy of PI3K pathway inhibitors as single agents or in combination with other drugs. The role of PI3K inhibitors for treating cancers in these mouse models and in human trials will be discussed.

Curing Acute Promyelocytic Leukemia without Chemotherapy

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Acute promyelocytic leukemia (APL) is a rare subset of leukemia characterized by a very aggressive clinical course. If unrecognized and not promptly treated, the disease might lead to fulminant death in few hours or days due to severe hemorrhages which frequently represent the first manifestation fo this leukemia and which may occur in internal organs (mostly brain and lungs). The advent of all-trans retinoic acid (ATRA) and its association with anthracycline chemotherapy in the early 90s have transformed APL into the most curable form of acute leukemia in adults with >80% of patients surviving in remission beyond 5 years, as shown by several multicenter studies conducted in Europe, China, Japan, the USA and Australia). Notably, instead of killing tumor cells ATRA is able to induce terminal differentiation of leukemic promyelocytes by selectively binding a nuclear receptor and releasing the maturation block of blasts. Notwithstanding these impressive advances, early death, disease relapse and the toxic effects of chemotherapy have represented important obstacles for the final cure and impacted negatively of patients outcome and quality of life. In more recent years, arsenic trioxide (ATO, an agent which had been used for centuries in both Western and Eastern world to cure several diseases) was shown to be highly effective in the treatment of APL and was therefore used in various combinations with chemotherapy and ATRA, initially as salvage therapy and thereafter in fron-line management of the disease. The Italian multicenter cooperative group GIMEMA recently completed a randomised study comparing standard ATRA and anthracyclines versus a chemotherapy-free ATRA and ATO protocol in newly diagnosed patients. Survival outcome analysis at 2 years showed that >98% of patients were alive and without disease in the ATO-ATRA arm with a statistically significant survival advantage over those receiving ATRA and antrhacyclines. Thus a treatment strategy only based on non-chemotherapy agents was shown for the first time to be potentially curative in acute leukemia, representing a model for targeted therapy in human cancer.

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T cell-engaging bispecific antibody constructs for treatment of leukemia

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Bispecific T cell-engaging (BiTE®) antibody constructs can transiently link tumor cells with otherwise inactive cytotoxic T cells for induction of potent redirected lysis of attached tumor cells. One example is blinatumomab (AMG 103), a CD19/-CD3-bispecific BiTE® antibody construct for the treatment of acute lymphocytic leukemia (ALL) and non-Hodgkin lymphoma (NHL), which is in advanced clinical development. Three other BiTE® antibody constructs, AMG 211/MEDI-565 (CEA/CD3-specific), AMG 110 (EpCAM/CD3-specific) and AMG 212/BAY2010112 (PSMA/CD3-bispecific) are in early clinical development for the treatment of solid tumors. A BiTE® antibody construct specific for CD33 and CD3, called AMG 330, is in formal preclinical development, and has shown high ex-vivo activity experimentally by redirecting autologous patient T cells for lysis of acute myelogenous leukemia (AML) blasts. Blinatumomab and all other BiTE® antibody constructs can activate T cells in a highly efficient manner that is strictly dependent on the presence of target cells, elicit serial lysis of target cells by T cells, induce T cell proliferation, and act at subnanomolar concentrations.

In a Phase 1 dose escalation study, blinatumomab elicited a complete response (CR)/partial response (PR) rate of 68% at a target dose level of 60 micrograms/ metersquared/day in relapsed or refractory NHL patients with, for instance, follicular, mantle cell lymphoma, or diffuse large B cell lymphoma. Initial phase 2 studies investigating monotherapy with blinatumomab in patients with minimal residual (N=20) or relapsed/relapsed (r/r) ALL (N= 36) revealed molecular/minimal residual disease response and complete hematologic response (CR and CRh) rates in the range of 69-80% at a target dose level of 15 micrograms/metersquared/day. A larger phase 2 study in r/r ALL patients (N=189) at a fixed dose of 28 µg per day confirmed a CR/CRh rate of 43%. Clinically most relevant safety events were neurological and related to cytokine release. An overview of the clinical program and insights into the mode of action, immunopharmacology, safety profile, and clinical activity of blinatumomab will be provided.

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(Re)Naissance of cancer immunotherapy: breaking tolerance

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Current strategies in immunotherapy are based on agents that can break tolerance. The most recognised class of immuno-oncology agents, checkpoint inhibitors, modulate pathways that switch off T-cell activity (i.e. inhibiting the inhibitor), or stimulate T-cell activity, thus potentiating antitumour responses and reducing tumour-induced immune suppression.These agents are recognised as breakthrough treatments for advanced melanoma; they also show considerable promise in other tumour types, particularly renal cell, lung and bladder cancer.

The most striking benefit of this approach is durable tumour control and survival. Mature data in thousands of patients have shown that around one in five patients treated with ipilimumab, an antibody that blocks cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), have the potential to survive for at least 3 years and up to 10 years, from treatment initiation. Similarly, treating patients with antibodies that block the programmed death-1 (PD1) receptor, or its ligand, PD-L1, has proved highly promising. Results of extended phase 1 trials evaluating two anti-PD1 antibodies (nivolumab and pembrolizumab) showed objective response rates of 30% to 50% in patients with advanced melanoma, with most responders having durable benefit of up to 2 years.

The first data on combining ipilimumab and nivolumab in melanoma indicate further improvement of outcome with > 90% survival at 1 year and >80% survival at 2 years. These numbers for advanced melanoma patients, only 4 years ago were 25% and 12% respectively, and thus we observe landscape changing developments.

Monoclonal antibodies against other immune checkpoint proteins, such as TIM3, LAG3, OX40, and KIRs are all being investigated in clinical trials and serve as potential components of a combination strategy. It is possible that once the 'brakes' elicited by the immune system have been released with one antibody, the inclusion of an agonistic antibody such as anti-OX40 could augment antitumour immune activity further. Potential combination strategies are not limited to the different immune checkpoint ligands and receptors. A rationale also exists for combining checkpoint inhibitors with other immunotherapeutic approaches, such as cytokines that increase the number of activated T-cells in circulation, or conventional cancer therapies, such as targeted kinase inhibitors, chemotherapy or radiotherapy. Drugs or radiotherapy schedules that lead to immunogenic cell death will provide a rational

basis to move forward in designing successful combination therapies involving different treatment modalities with immunotherapy.
Durable tumour control and long-term survival depend on harnessing the power of the immune system. Data with agents that block CTLA-4, PD1 and other checkpoint proteins are not only providing a benchmark against which future therapies will be compared, but are stimulating interest in alternative sequencing or smart combination approaches that could improve outcomes even further. In changing the treatment landscape, immuno-oncology advances offer renewed hope to patients with advanced melanoma at present and to patients with other solid tumours in the near future.

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Poster Abstracts

1

Enhancer cooperativity as a novel mechanism underlying the transcriptional regulation of E-cadherin during mesenchymal to epithelial transition

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Epithelial-mesenchymal transition and mesenchymal-epithelial transition (MET) exemplify crucial steps during embryogenesis and tumorigenesis. Induction of marked changes in gene expression and cell features is reflected by modulation of Cdh1 (E-cadherin) expression. We show that Cdh1 activity during MET is governed by two enhancers at +7.8 kb and at +11.5 kb within intron 2 that are activated by binding of Grhl3 and Hnf4α, respectively. Recruitment of Grhl3 and Hnf4α to the enhancers is crucial for activating Cdh1 and accomplishing MET in normal mouse mammary gland cells. Moreover, the two enhancers cooperate via Grhl3 and Hnf4α binding, induction of DNA-looping and clustering at the promoter to orchestrate E-cadherin re-expression. Our results provide novel insights into the cellular mechanisms whereby cells respond to MET signals and re-establish an epithelial phenotype by enhancer cooperativity. A general importance of our findings including MET-mediated colonization of metastasizing tumor cells is suggested.

2

Inositol trispyrophosphate (ITPP) and its anti-hypoxic potential in colorectal metastases of the liver

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The hypoxic tumor response not only promotes angiogenesis, but also a number of other processes associated with malignant behavior. Therefore, inhibition of hypoxia rather than angiogenesis may be a potent anticancer strategy. The recently designed molecule ITPP promotes oxygen release from hemoglobin under hypoxic conditions. We assessed whether ITPP can inhibit hypoxia and improve outcome in a mouse model of colorectal cancer (CRC) liver metastasis. Two syngeneic orthotopic mouse models of hepatic CRC metastasis were established by selective portal vein injection of CRC cells. Small animal magnetic resonance imaging was used to follow metastatic development in vivo. Oxygen dissociation kinetics from hemoglobin were determined by tonometry. Localization of hypoxic areas was achieved by pimonidazole staining on histological sections. Mice treated with ITPP had a significant survival benefit along with a reduced tumor burden. ITPP had an antihypoxic effect as demonstrated by pimonidazole staining, HIF down-regulation, Warburg inhibition, inflammatory changes, the normalization of systemic angiogenesis/metastasis markers, and a reduced cancer cell invasiveness. Notably, the ITPP effects were maintained following cessation of treatment. Combining ITPP with standard chemotherapy prolonged survival by three times and was superior to chemotherapy plus targeted anti-angiogenic therapy. In conclusion, ITPP is a potent inhibitor of the hypoxic tumor response. Its anti-hypoxic action favors a more benign tumor phenotype that is accompanied by reduced tumor invasiveness and increased survival. ITPP appears to act synergistically with cytotoxic agents. A planned Phase Ib/IIa clinical trial will reveal whether ITPP holds promise as a novel anti-hypoxic agent.

3

Benzene-poly-carboxylic acid complex, a novel anti-cancer agent induces apoptosis in human cancer cells

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Benzene-poly-carboxylic acid complex (BP-C1) is a novel anti-cancer complex of benzene-poly-carboxylic acids with a very low concentration of cis-diammineplatinum (II) dichloride. In the present study, the effect of BP-C1 on growth of human breast cancer cells and its molecular mechanism of action were tested. Cell viability of MCF-7 and T47D cells was detected by XTT assay and apoptosis was detected by Flow Cytometry and by annexin V/ FITC/PI assay. Caspases were detected by western blot analysis and gene expression was measured by using the Applied Biosystems® TaqMan® Array Plates. Exposure of the human breast cancer cells to BP-C1 for 48h is not toxic and significantly ($P<0.001$) reduced cell viability, induced apoptosis and activated caspase 8 and caspace 9. Moreover, gene expression experiments indicated that BP-C1 increased the expression of pro-apoptotic genes (CASP8AP1, TNFRSF21, NFkB2, FADD, BCL10 and CASP8) and lowered the level of mRNA transcripts of inhibitory apoptotic genes (BCL2L11, BCL2L2 and XIAP). Similarly, BPC-1 significantly reduced cell viabilty of human prostate, pacreatic, colon, head and neck and colon cancer cells. These findings may lead to the development of new therapeutic strategies for treatment of human cancer using BP-C1 analog.

4

Co-targeting VEGF and TGF-β in glioma models in vitro and in vivo

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Glioblastoma is the most common form of malignant brain tumor in adults. Despite surgery, radiotherapy and concomitant chemotherapy, patients suffering from this tumor have a very poor prognosis with a median survival time of 16 months in clinical trial populations[1]. The highly invasive phenotype, together with its formidable adaptive nature, makes this tumor one of the most challenging to treat. Current standard of care is hardly effective and the main effort in the field consists of finding new and better therapies based on our increasing knowledge of the complex signaling pathways that drive this devastating disease. Recently, experience with a humanized monoclonal antibody targeting vascular endothelial growth factor A (VEGF-A), bevacizumab, suggested a potential benefit through anti-angiogenic therapies. Although recent controlled clinical trials yielded disappointing results regarding overall survival, bevacizumab remains a molecule of great interest, particularly for developing combination therapies that could overcome acquired drug-resistance, which is presently unavoidable. Transforming growth factor-β (TGF-β) is a master molecule that regulates, through the canonical and non-canonical arms of the signaling pathway, changes in cell phenotype that in gliomas have been linked with immunosuppression, invasiveness, angiogenesis and poor overall survival. VEGF and TGF-β are both pivotal molecules in glioma growth, progression and spread and act by regulating important oncogenic properties. Our previous work, and the observation that overlapping pathways are regulated through these two molecules, accentuates the hypothesis involving an intense cross-talk. Hence, we are investigating whether primary or acquired resistance to anti-VEGF therapy is mediated by TGF-β. In order to achieve this goal we are exploring strategies to co-inhibit VEGF and TGF-β in syngeneic, experimental tumor mouse models.

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5

Identification of a drug resistance signature by proteome analysis

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Use of the FDA-approved BRAF inhibitor vemurafenib has resulted in outstanding clinical response rates in patients with melanoma, but early resistance is common. Understanding the pathologic mechanisms of drug resistance, and identification of effective therapeutic alternatives, are key scientific challenges in relation to melanoma. Using proteomic techniques including shotgun analysis and 2D-gel electrophoresis, we identified a comprehensive signature of the vemurafenib-resistant M24met in comparison to the vemurafenib-sensitive A375 melanoma cell line. The resistant cells were characterized by loss of differentiation, induction of transformation, enhanced expression of the lysosomal compartment, increased potential for metastasis, migration, adherence and Ca2+ ion binding, enhanced expression of MAPK pathway and extracellular matrix proteins, and epithelial-mesenchymal transformation. The main features were verified by shotgun analysis with QEXACTIVE orbitrap MS, electron microscopy, lysosomal staining, western blotting, and adherence assay in a VM-1 melanoma cell line with acquired vemurafenib resistance. Based on the resistance profile, we were able to successfully predict that a novel resveratrol-derived COX-2 inhibitor, M8, would be active against the vemurafenib-resistant but not the vemurafenib-sensitive melanoma cells. As a next step, we propose to analyse the protein signature of primary sensitive and resistant melanoma cells and patient tissue. Employing high-throughput methods for cell line and drug characterization may offer a new way to identify key features of vemurafenib resistance, facilitating the design of effective rational therapeutic alternatives.

6

Incomplete cytokinesis and re-fusion of small mononucleated Hodgkin cells lead to giant multinucleated Reed-Sternberg cells

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The multinucleated Reed-Sternberg (RS) cells are pathognomonic for classical Hodgkin lymphoma (HL) and their presence is a requirement for diagnosis. However, the development of these giant tumor cells is subject to controversy. It has been postulated that RS cells arise from mononucleated Hodgkin cells via endomitosis. Conversely, continuous single cell tracking of HL cell lines by long-term time-lapse microscopy suggested that cell fusion is the main route of RS cell formation. In contrast to growth-induced formation of giant Hodgkin cells, fusion of small mononuclear cells followed by size increase may give rise to giant RS cells. Importantly, we were among very few to observe fusion of cells originating from the same ancestor, termed re-fusion. In the majority of cases, re-fusion of daughter cells was preceded by

an incomplete cytokinesis, evidenced by sighting of a microtubule bond between the cells. We confirm at the level of individual tracked cells that giant Hodgkin and RS cells have little proliferative capacity, this result further identifying small mononuclear Hodgkin cells as the proliferative compartment of the HL tumor clone. In addition, sister cells showed a shared propensity for re-fusion, which provides evidence of early RS cell fate commitment. Thus, RS cell generation is neither due to cell fusion of unrelated Hodgkin cells nor to endomitosis, but is mediated by re-fusion of daughter cells that have undergone mitosis. This surprising finding indicates the existence of a novel mechanism for the generation of multinuclear RS cells, which might have implications beyond HL, as RS-like cells are frequently observed in several other lymphoproliferative diseases.

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7

Tumor-infiltrating HLA-matched CD4 T cells lack rosette formation and show reduced reactivity against Hodgkin lymphoma

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Hodgkin lymphoma (HL) presents with a unique histological pattern. Pathognomonic Hodgkin and Reed-Sternberg (HRS) cells account for less than 1% of the tumor mass and are embedded in a reactive infiltrate mainly comprised of CD4 T cells. To escape anti-tumor immunity, HRS cells are thought to modulate the surrounding cells, inducing an immunosuppressive tumor microenvironment. In contrast to this perspective, we identified a strong anti-tumor potential exhibited by CD4 T cells against HRS cells in vitro and in vivo. Interestingly, we were able to visualize adhesion complexes between HRS and CD4 T cells comparable to conventional immunological synapses. Further studies revealed that tumor-cell killing was based on recognition of allogeneic major histocompatibility complex class II (MHC-II), as CD4 T cells from MHC-II-compatible donors did not develop anti-tumor potential. However, gene expression profiling of co-cultured HRS cells, as well as tumor infiltration of matched CD4 T cells, indicated cellular interactions probably resembling the situation in situ. Therefore, we introduced an HRS-cell specific chimeric antigen receptor into matched CD4 T cells, restoring their potential to reject HRS cells. Our work gives singular insight into the crosstalk between HRS and CD4 T cells, indicating the latter to be perfectly suited for a novel immunotherapeutic approach to HL.

8

EWS/FLI1 transcription is modulated by the PI3K pathway via SP1 in Ewing Sarcoma

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Ewing sarcoma (ES) is the second most frequent bone cancer in childhood and is characterized by the presence of the balanced t(11;22)(q24;q12) translocation in 85% of cases, generating a dysregulated transcription factor EWS/FLI1 which is essential for ES development and tumor cell maintenance, through inappropriate regulation of target genes, crucial for the fully malignant phenotype. Therefore, EWS/FLI1 represents an attractive therapeutic target. Screening of a small library of 153 targeted compounds identified inhibitors of PI3K pathway as primary modulators of EWS/FLI1 activity and also, surprisingly, modulators of its transcription. Treatment of four different ES cell lines with BEZ235 (PI3K-mTOR inhibitor) resulted in down regulation of EWS/FLI1 expression by 50% with subsequent modulation of the expression of target genes. Analysis of the EWS/FLI1 promoter using various deletion constructs identified two 14bp minimal elements as important for EWS/FLI1 transcription. Based on in silico prediction, we were able to predict

4 transcription factor candidates to bind this region and to be responsive to PI3K regulation. Among these, SP1 was identified as modulator of EWS/FLI1 transcription by siRNA. Various approaches, such as qRT-PCR, Western blotting and immunofluorescence, confirmed a reduction of EWS/FLI1 levels in Ewing cells when PI3K$\alpha\gamma\delta$ and SP1 were depleted; electrophoretic mobility shift and chromatin immunoprecipitation assays confirmed that SP1 is indeed binding to EWS/FLI1 promoter in the minimal element previously identified. In summary, our results provide particular initial insights on EWS/FLI1 transcriptional regulation, an area that has not been previously investigated, and offer a molecular explanation for the known sensitivity of ES cell lines to PI3K inhibition.

9

Synergistic anti-tumor effects on human breast cancer cells by the mevalonate pathway inhibitors atorvastatin and zoledronic acid

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Breast cancer is the most frequent malignancy in women and often leads to osteolytic bone metastases. Amino-bisphosphonates are anti-resorptive drugs and currently standard of care for affected patients. Similarly to statins, they inhibit the mevalonate pathway, which is essential for the farnesylation and geranylgeranylation of proteins. Here, we demonstrate that this inhibition by atorvastatin and zoledronic acid evokes synergistic anti-tumor effects in human breast cancer cells. Human triple-negative MDA-MB-231 breast cancer cells were treated with 1 µM atorvastatin and 10 µM zoledronic acid. Strikingly, whereas individual treatments only elicited weak effects, the combination induced a three-fold increase of caspases 3 & 7 activation and DNA fragmentation, as well as decreasing viability of treated cells by 60%. The synergistic effects were additionally emphasized by the induction of caspase 3 and poly ADP ribose polymerase cleavage and a significant reduction in expression of the anti-apoptotic genes B-cell lymphoma 2 (BCL-2) and survivin (SVV) by 50%. Furthermore, the treatments significantly reduced the expression and secretion of interleukins 6, 8 and 11, as well as of Dickkopf-1, these genes major players in the vicious cycle of osteolytic bone metastases. Finally, supplementing the cells with either farnesylpyrophosphate or geranylgeranylpyrophosphate (GGPP) upon the combination treatment clearly revealed that only GGPP completely abrogated the synergistic anti-tumor effects. These observations clearly demonstrate that geranylgeranylation is indispensable for the survival of MDA-MB-231 cells. Our results indicate the synergistic use of mevalonate pathway inhibitors as a new attractive therapeutic approach for the treatment of breast cancer-induced bone metastases.

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10

Complement is a central mediator of radiotherapy-induced tumor-specific immunity

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Radiotherapy induces irreversible DNA damage, but recent data suggest that concomitant immune stimulation is an integral part of the therapeutic action of ionizing radiation. How radiotherapy supports tumor-specific immunity is insufficiently understood, although several pathways have been suggested to play a role. Here we show that tumor cell death resulting from radiotherapy is accompanied by transiently-activated complement in murine and human tumors. Moreover, local production of pro-inflammatory anaphylatoxins C3a and C5a was crucial to the tumor response to radiotherapy and concomitant stimulation of tumor-specific immunity. Dexamethasone, frequently given during radiotherapy treatment, limits complement activation and its effectiveness. Overall, our findings identify anaphylatoxins as key players in radiotherapy-induced tumor-specific immunity and therapeutic efficacy.

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11

HPV E6: The Achilles' Heel of Cervical Cancer?

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Cervical cancer is caused by high-risk human papillomaviruses (HPV). The HPV E6 and E7 oncoproteins maintain the malignant phenotype of HPV-positive cancer cells. While E7 acts as a proliferation stimulus, E6 inhibits apoptosis induction by forming a trimeric complex with the cellular E6AP ubiquitin ligase and p53, ultimately leading to p53 degradation. This scenario indicates that E6 represents an attractive therapeutic target. Here, we introduce a novel peptidic inhibitor, pep11**, which specifically binds and inhibits HPV16E6, leading to p53 restoration and apoptosis in HPV16-positive cells (1). To identify the pep11**-binding site within HPV16E6, we have performed nuclear magnetic resonance interface studies and comprehensive intracellular interaction studies. Binding of pep11** to HPV16E6 was compared with that of E6APpep, a short peptide corresponding to the E6-binding motif of E6AP. Interestingly, many amino acid residues of HPV16E6 contacted by pep11** are also involved in E6APpep-binding, indicating that pep11** binds to the recently identified E6AP-binding pocket (2). Consistent with this understanding, fluorescence polarization studies show that pep11** competes with E6APpep for binding to HPV16E6. In vitro and intracellular binding studies both indicate that pep11** binds to HPV16E6 with a higher affinity than E6APpep. Interpretation of the data from the HPV16E6/pep11** interaction allowed the rational design of single amino acid exchanges in HPV18E6 and HPV31E6 that enabled their binding to pep11**. This is strong evidence for our model of the pep11**/E6 interaction (3). Taken together, this study provides to our knowledge the first experimental evidence that HPV16E6 is druggable by targeting the E6AP binding pocket, opening new possibilities for rational, structure-based drug design.

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12

Tumor-associated stromal cells increase malignancy of human colorectal cancers triggering EMT induction

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During tumor formation, the tissue microenvironment is transformed in an "altered" niche, composed of non-malignant cells, which influences cancer behavior. Tumor-associated stromal cells (TASC) are the prominent stromal elements in the tumor. The differentiation of TASC from different sources, such as resident stromal cells or recruited mesenchymal stromal cells, is mediated by crosstalk with the tumor. TASC produce extracellular matrix proteins, chemokines, and factors which affect tumor proliferation, invasiveness and survival. Emerging data suggest that TASC play a role in response to

therapy by influencing cell-cell interactions and suppression of anti-tumor immune responses. Moreover, physical contact between TASC and tumor supports cell survival via anti-apoptotic pathways or epithelial-to-mesenchymal transition (EMT). In this scenario, the specific role played by the stromal component and the processes induced on colorectal cancer (CRC) cells remain to be fully elucidated. For this purpose, we have addressed phenotypic and functional characterization of TASC in vitro and we have analyzed TASC-mediated effects on CRC development and progression in vivo. Our results indicate that TASC comprise a multipotent subpopulation that is able to differentiate into adipogenic and osteogenic lineages. After co-culture with CRC cells they express membrane-bound TGF-β, through which they are able to initiate EMT and induce a more invasive phenotype in tumor cells. Upon subcutaneous injection in NOD/SCID mice, tumor cells co-cultured with TASC show more aggressive tumorigenicity and the tumor masses developed are characterized by a higher vessel density. Thus our data show that the stromal component of CRC increases the tumor cells malignancy triggering EMT induction.

13

Bioreactor-engineered cancer tissues mimic phenotypes, gene expression profiles and drug resistance patterns observed in xenografts and clinical specimens

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Anticancer compound screening on bi-dimensional (2D) cell cultures poorly predicts in vivo performance and requires validation using xenografts. We addressed the generation of tri-dimensional (3D) tissue-like structures amenable to drug sensitivity testing from cultured cancer cells. Tumor cell lines were cultured in 2D, on collagen scaffolds in static conditions or in perfused bioreactors, or injected subcutaneously in immunodeficient mice. Gene expression profiles were evaluated by whole genome RNA-sequencing. Drug sensitivity was comparatively analyzed in vitro and in vivo and drug resistance-related markers expression was explored in samples from patients undergoing neo-adjuvant chemotherapy. Perfused 3D (p3D) culture allowed more homogeneous scaffold seeding than static 3D cultures and significantly higher cell ($p<0.0001$) proliferation. Using colorectal cancer (CRC) HT-29 cells as model, we observed that resulting tissue-like structures exhibited morphology and phenotypes similar to xenografts. Transcriptome analysis revealed a high correlation between xenografts and p3D cultures ($r=0.985$). 5-Fluorouracil (5-FU) treatment induced apoptosis and significant BCL-2, TRAF1, and c-FLIP gene down-regulation in monolayers, but only nucleolar stress in perfused cells and xenografts, and reduced by 55% cell numbers in 2D but not in p3D cultures or xenografts. Conversely, the BCL-2 inhibitor ABT-199 induced cytotoxic effects in p3D but not in 2D cultures. Following neo-adjuvant 5-FU-based chemotherapy, tumor cells from 14 of 26 unresponsive or partially responsive (Dworak 0-2) patients were found to express to variable extent BCL-2, consistent with the trend captured by p3D cultures. We conclude that p3D cultures efficiently mimic functional features observed in CRC xenografts and clinical specimens.

14

Establishment of the culture condition to maintain the Human Colorectal Cancer microenvironment by using a tri-dimensional perfusion bioreactor

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The use of tri-dimensional (3D) systems based on human tissue may produce drug responses more predictive of human cancers than non-human models and so could be an innovative and efficient tool able to bridge the gap between bi-dimensional (2D) cultures and animal model for the development

of new therapeutic strategies. We demonstrated the possibility of using a perfusion-based bioreactor system to maintain alive and proliferating freshly surgical excised colorectal cancer (CRC) tissue fragments in porous 3D collagen type-1 scaffolds for 10 to 20 days. CRC tissues were effectively expanded in perfused 3D cultures in 7 out of 12 cases where, gene profiles of expanded tumor tissues suggested a heterogeneous tissue composition, as indicated by the expression of EpCAM, CD90, CD8, CD16 and Foxp3 genes. Phenotypic analysis confirmed that expanded tissues included epithelial (EpCAM+) and stromal cells (Vimentin+). Evidence of tumor cell proliferation was provided by Ki67 staining. Furthermore, infiltrating CD4+ and CD8+ lymphocytes were consistently identified within cultured tumor fragments. Taken together, our results indicate that culture of primary tumor fragments within perfused bioreactors can be successfully achieved over a short-time period in a reproducible manner allowing the preservation of the diverse cellular components of the CRC specimens for possible personalized medicine purposes of epithelial and interstitial cells. These ex-vivo generated tissues might mirror features of the original tumor more effectively than 2D or 3D static cultures, and of patient-derived xenografts, thus possibly representing useful tools for the evaluation of sensitivity to chemotherapies or new targeted treatments.

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CD70/CD27-signaling is an independent adverse prognostic marker and novel therapeutic target for acute myelogenous leukemia

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Acute myeloid leukemia (AML) is a very heterogeneous hematological neoplasm that is characterized by the accumulation of immature myeloid blasts in the blood and bone marrow (BM). Recent advances in genetics have shed light on the pathogenesis of AML; however, treatment options are still limited and prognosis is dismal with a 5-year overall survival of less than 30%. Because AML incidence peaks in the elderly who are usually ineligible for intensive therapeutic regimens, additional treatment options for older patients are urgently needed. Here, we demonstrate that AML cell lines and blasts from blood and BM of newly diagnosed AML patients co-express the tumor necrosis factor superfamily ligand-receptor pair CD70 and C D27. CD70/CD27-signaling induced stem cell signature and proliferation-promoting pathways in primary AML blasts, particularly the canonical Wnt pathway. Blocking CD70/CD27-interactions by monoclonal antibodies reduced Wnt-signaling, inhibited AML cell growth and colony formation in vitro and significantly prolonged survival in murine AML xenografts. Soluble CD27, a marker for the extent of CD70/CD27-interactions in vivo, was considerably increased in the sera of newly diagnosed AML patients and correlated with poor overall survival independently of age or AML risk group. Blocking CD70/CD27-signaling may represent an attractive novel therapeutic strategy for AML.

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Tyrosine kinase inhibitor-induced CD70 expression mediates drug resistance in chronic myelogenous leukemia stem cells by activating Wnt-signaling

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Chronic myelogenous leukemia (CML) originates from leukemia stem cells (LSCs) harboring the oncogenic BCR-ABL1 tyrosine kinase. BCR-ABL1 stabilizes β-catenin, a central component of the Wnt pathway that is crucial for LSC maintenance. Treatment with tyrosine kinase inhibitors (TKIs) reduces CML often to molecular remission; however, LSCs are insensitive to TKIs

despite BCR-ABL1 inhibition, persist long-term and ultimately lead to disease relapse. Here, we demonstrate that TKI treatment increases the expression of the tumor necrosis factor superfamily ligand CD70 in CML stem/progenitor cells. TKI-mediated BCR-ABL1 inhibition down-regulated microRNA-29 levels, resulting in reduced CD70 promoter DNA methylation and increased expression of the transcription factor specificity protein 1. CD70 triggered signaling via its receptor CD27, leading to compensatory Wnt activation. Combined inhibition of BCR-ABL1 and CD70/CD27-signaling synergistically reduced Wnt-signaling and eradicated leukemia cells in vitro. In addition, combination therapy using TKIs and CD70 blocking monoclonal antibodies effectively eliminated CD34+ CML stem/progenitor cells in murine xenografts and LSCs in a murine CML model. Therefore, CD70 up-regulation and consecutive CD27-signaling leading to compensatory Wnt activation is an important targetable TKI resistance mechanism of CML LSCs.

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Tissue Engineering of melanoma in a humanized model in vivo

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To overcome the lack of effective therapeutics for aggressive melanoma, new research models closely resembling the human disease are required. Here we report the development of a fully orthotopic, humanized in vivo model for melanoma, faithfully recapitulating human disease initiation and progression. To this end, human melanoma cells were seeded into engineered human dermo-epidermal skin substitutes. Transplantation onto the back of immunocompromised rats consistently resulted in the development of melanoma, displaying the hallmarks of their parental tumors. Importantly, all initial steps of disease progression were recapitulated, including the incorporation of the tumor cells into their physiological microenvironment, transition of radial to vertical growth, and establishment of highly vascularized, aggressive tumors with dermal involvement. Because all cellular components can be individually accessed using this approach, it allows manipulation of the tumor cells, as well as of the keratinocyte and stromal cell populations. Therefore, in one defined model system, tumor cell-autonomous and non-autonomous pathways regulating human disease progression can be investigated in a humanized, clinically relevant context.

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Validating nanoparticles for cancer immunotherapy: the one plate/one day method

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The main advantages to use nanoparticle as a drug carrier for cancer immunotherapy are first, control over the timing and distribution of drugs; second, ability to stabilize, protect and solubilize the drug inside the organism and third, the possibility to target specific cell types. Because nanoparticles represent a promising drug delivery system, we are investigating the use of nanoparticles for the immunotherapy of tumors. However, although many types of highly diverse nanoparticles and micelles are well characterized, we and others have shown that their characteristics in cell culture are difficult to predict. In view of this heterogeneity, we have established a new standardized operating protocol (SOP) to validate nanoparticles for biological use. Three parameters are tested in parallel: toxicity is measured by an MTT assay and flow cytometry, immunogenicity is quantified by surface activation markers and by cytokine secretion, whereas cellular uptake is determined by flow cytometry and immunofluorescence. Successful nanoparticles must have low cytotoxicity and must be taken up by immune cells. Immunogenicity is desirable for carriers . The main force of this SOP is the fact that all these data are obtained in one experimental day in only one plate. This permits an economy of nanoparticles, the protocol is highly reproducible and allows comparison between different types of nanoparticles. This project is part of the recently established National Center of Competence in Research for Bio-inspired Stimuli-Responsive Materials (www.bioinspired-materials.ch)

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Discontinuation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis

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Here we report a paradoxical effect of the CC chemokine ligand 2 (CCL2) in metastatic breast cancer. Secretion of CCL2 by mammary tumors recruits CCR2-expressing inflammatory monocytes to primary tumors and metastatic sites, and CCL2 neutralization in mice inhibits metastasis(1) by retaining monocytes in the bone marrow. Surprisingly, interruption of CCL2 inhibition leads to an overshoot of metastases and accelerates death. This is the result of monocyte release from the bone marrow, enhancement of cancer cell mobilization from the primary tumor, as well as blood vessel formation and increased proliferation of metastatic cells in the lungs in an IL-6/VEGF-dependent manner. Notably, inhibition of CCL2 and IL-6 dramatically reduced metastases and increased survival of the animals. CCL2 has been implicated in various neoplasias and adopted as a therapeutic target(1-3). However, our results call for caution when considering anti-CCL2 as monotherapy in metastatic disease and highlight the tumor microenvironment as a critical determinant of successful anti-metastatic therapy.

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Intracellular serotonin regulates cytoskeletal remodeling at the base of pancreatic acinar-to-ductal-metaplasia formation

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Pancreatic ductal adenocarcinoma (PDAC) is the most frequent and lethal pancreatic cancer initiated by persistent lesions called acinar-to-ductal metaplasias (ADMs). Noteworthy, ADM formation mainly depends on cytoskeletal remodeling of acinar cells (1). After showing that intracellular serotonin (5-hydroxytryptamine, 5-HT) regulates actin re-arrangements in acinar cells (2), we investigated whether 5-HT regulates the cytoskeletal remodeling critical for ADM and pre-malignant lesion formation. Several biochemical and imaging approaches, acinar and human pancreatic cancer cell lines, primary pancreatic fibroblasts, an in vitro 3D culture model of ADM formation and 5-HT deficient mice were utilized to identify the molecular mechanisms at the base of 5-HT-dependent cytoskeletal remodeling. The therapeutic potential of selective 5-HT transporter inhibitors (SERTi) was evaluated in vivo in transgenic mice harbouring KrasG12D mutation which spontaneously form ADM and pre-malignant lesions. In vivo and in vitro analyses showed that intracellular 5-HT is required for Rac1 activation, a key regulator of cytoskeletal remodeling. Inhibition of 5-HT transport with SERTi in vitro inhibited ADM formation, reduced cell migration, increased cell adhesion and perturbed lipid metabolism without reducing cell viability. Importantly, in vivo treatment of KrasG12D mice with SERTi significantly reduced the formation of ADM and pre-malignant lesions without associated drug toxicity. We conclude that cytoskeletal remodeling regulated by intracellular 5-HT is critical for the formation of ADM lesions. Given the role of ADMs as precursors of PDAC, we propose a novel therapeutic strategy which might complement current PDAC treatments.

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A novel connection between CtIP and BRCA1/BARD1 emerges through functional RNAi screening

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Human CtIP has been considerably recognized for its role in the repair of DNA double-strand breaks by homologous recombination. Furthermore, increasing evidence implicates CtIP as a critical factor for the maintenance of genome stability owing to its roles in transcription, the DNA damage response and cell cycle checkpoint control. In order to explore the molecular network of CtIP involved in the maintenance of genome stability and to investigate functional relationships of CtIP, we reverted to the systematic analysis of synthetic genetic interactions of CtIP by RNA interference (RNAi)-based screening. Intriguingly, we found the BRCA1-associated RING domain protein 1 (BARD1) among our strongest hits for aggravating synthetic interactions with loss of CtIP functions. BARD1 is the main interaction partner of the tumor suppressor BRCA1 and the BRCA1/BARD1 complex is thought to contribute to genome stability by facilitating DSB repair via the HR pathway. Follow-up analysis revealed that simultaneous disruption of BARD1 and CtIP decreases cell viability to a larger extent than co-depletion of BRCA1 and CtIP suggesting that BARD1 and CtIP might have a functional connection beyond the BRCA1/BARD1 heterodimer. Our data indicates that prolonged impairment of BARD1 and CtIP delays cell proliferation and increases cell death. Furthermore, cells defective for BARD1 and CtIP display an aberrant response to replication stress. Collectively, our current model suggests that CtIP and BARD1 cooperate to maintain faithful DNA replication and genome stability. Loss of function of BARD1 and CtIP might cause replication stress, which could give rise to chromosome instability and cancer.

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Dissecting the role of the hematopoietic oncoprotein FOXP1 in diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most commonly occurring form of non-Hodgkin lymphomas and accounts for approximately 30% of all B-cell lymphomas. We have previously shown that forced expression of the microRNA miR-34a in cell lines and xenograft models of DLBCL has tumor suppressive effects. These effects are elicited by the induction of apoptosis, partly by repressing the established miR-34a target FOXP1 (1), a transcription factor with oncogenic properties in DLBCL. The downstream targets of FOXP1 in this model are currently unknown. Using siRNAs targeting FOXP1 expression, we were able to show that FOXP1 knockdown induces apoptosis in different DLBCL cell lines, thereby phenocopying the effects of forced miR-34a expression. Using ChIP-Seq of DLBCL cell lines expressing various FOXP1 levels and RNA-Seq after FOXP1 knockdown, we identified direct and indirect FOXP1 target genes. Thus we were able to show that the sphingosine 1-phosphate receptor 2 (S1PR2) is repressed by FOXP1. After FOXP1 knockdown, mRNA levels of S1PR2 were increased and we were able to identify two FOXP1 binding sites, 2.5kb and 5kb upstream of the FOXP1 promoter. Moreover, forced expression of S1PR2 induced apoptosis in DLBCL cell lines, thereby phenocopying the FOXP1 knockdown effects. Using two microarrays we were able to demonstrate an inverse correlation between FOXP1 and S1PR2 expression in DLBCL patients. Patients expressing low levels of S1PR2 had a more dismal survival prognosis. Using this approach we identified S1PR2 as a novel FOXP1 target, whose repression possibly plays a role in DLBCL oncogenesis.

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Mechanisms regulating calretinin expression in malignant pleural mesothelioma

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Calretinin (CR, CALB2) is a calcium binding protein used as a diagnostic and prognostic marker in malignant pleural mesothelioma (MPM). Currently, nothing is known on its regulation in MPM cells. Our aim was therefore to investigate mechanisms regulating CR expression in MPM. Characterization of CR levels in different MPM cell lines showed that the mRNA levels correlate with protein expression. Hence, several genomic sequences surrounding +1 (800 bp upstream and 58 bp downstream of the CR gene, CALB2, transcription start site (TSS)) were cloned into the pGL3-b reporter vector. The sequence of 160 bp upstream of the TSS was sufficient to drive CR expression. Having defined the minimum sequence to drive CR expression, consensus sequences of potential functional transcription factors were identified and mutated. By using site-directed mutagenesis and electrophoretic mobility shift assay, we observed that NRF-1 and E2F binding to the CALB2 promoter might be essential. This is consistent with the observation that CR expression changed during cell cycle. Treatment with the hypomethylating agent 5-Aza-CdR did not change CR expression and analysis of TCGA database confirmed that CALB2 promoter is not hypermethylated in tumors compared with non-tumor tissue. In addition, CALB2 promoter activity was up-regulated upon ionomycin treatment. Intracellular Ca2+ levels were monitored with the recombinant fluorescent Ca2+ indicator GCaMP3. The selected ionomycin concentration evoked a transient Ca2+ signal that reflected the depletion of estrogen receptor stores; ionomycin also increased CR expression and was associated with increased activation of ERK and CREB.

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Identification of genomic changes in clonal tumor populations of bladder cancer recurrences

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Genomic aberrations accumulate during cancer progression and provide a growth advantage to tumor cells. We interrogated the clonal composition of bladder cancer and genomically analyzed the clonal populations within these tumors with the aim of assessing the dynamics of these cancer populations and their mutations over time. A sample cohort consisting of formalin-fixed paraffin-embedded tumor specimens from 14 patients with bladder cancer was selected; each of these patients with at least two time points and a minimal time interval of one year between the two biopsies. Nuclei were extracted from the tissues and stained with DAPI or Draq7. Tumor populations were sorted according to their ploidy by the BD Influx or ARIA III FACS. DNA from sorted populations was subjected to whole genome high resolution comparative genomic hybridization microarrays. Many of the bladder cancers analyzed thus far are composed of multiple clonal tumor populations. Pure sorted bladder cancer material allowed the detection of high and focal amplicons, as well as of homozygous deletions at high resolution. Distinct clonal tumor populations from the same biopsies revealed shared as well as population-specific genomic aberrations. Genomic profiling of sorted tumor populations thus allowed for the dissection of intratumoral genomic heterogeneity in bladder cancer. The further bioinformatic analysis and the application of next-generation sequencing data of these sorted populations will provide us with the possibility of inferring the clonal evolution of each bladder cancer in this study. Our results will help to understand the mechanisms of bladder cancer recurrence and progression.

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The regulatory role of non-hematopoietic bone marrow cells in steady-state and during inflammation

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Hematopoiesis is a hierarchically organized system that produces all types of blood cells. This process is taking place within the bone marrow (BM) and the hematopoietic cells are surrounded by different types of supportive cells called the BM microenvironment. We set out to study how the BM microenvironment supports the hematopoietic system in adaptation to inflammation. To this end, wild-type (WT) mice were stimulated with lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid to mimic gram-negative bacterial or viral infection, respectively. Using CD45 and Ter119 to exclude hematopoietic cells, mesenchymal stromal cells, CXCL12-abundant reticular cells (CARs), and endothelial cells can be identified by flow cytometry based on their different expression of Sca1, CD31 and CD140b. These various cell types were isolated and their gene expression profiles were determined and compared to steady-state WT mice using microarray analysis. We observed that interleukin 6 (IL6) is significantly and specifically up-regulated during LPS stimulation by the cell population enriched for CARs. This was validated using quantitative PCR and changes in IL6 protein production were also analyzed by measuring IL6 protein levels at different time points after LPS injection. Future experiments using the Cre-loxP system will be aimed at determining which BM cell population is the main source of IL6. In summary, our preliminary data shows that IL6 is mainly expressed following LPS treatment by BM resident CAR cells suggesting that IL6 may play a role in regulating hematopoiesis during bacterial infection.

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Radiolanthanides for radioimmunotherapy: Terbium-161 outperforms lutetium-177 in tumour growth inhibition by 82%

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The L1 cell adhesion molecule (L1CAM) is considered a valuable target for therapeutic intervention in different types of cancer. Recent studies have shown that anti-L1CAM radioimmunotherapy (RIT) with 177Lu-labelled internalising mAb chCE7 was effective in the treatment of human ovarian cancer [1,2]. In this study we directly compared the therapeutic efficacy of anti-L1CAM RITs with the radiolanthanides lutetium-177 and the potential alternative terbium-161 against human ovarian cancer under equitoxic conditions. Thereby, 161Tb emitts 16 times more auger- and conversion electrons than its similar counterpart 177Lu. In vivo therapy- and bio-distribution studies were performed in nude mice bearing subcutaneous IGROV1 ovarian cancer xenografts. For therapy purposes mice were injected with equitoxic activities of 6 MBq 177Lu-DOTA-c hCE7 (50% maximum tolerated activity, MTA) or 5 MBq 161Tb-DOTA-chCE7 (50% MTA). Dose escalation studies revealed a decreased MTA for 161Tb-DOTA-chCE7 (10 MBq) compared to 177Lu-DOTA-chCE7 (12 MBq). Average relative tumour volume of the 161Tb-therapy group was significantly reduced compared to that in mice receiving the 177Lu containing RIT ($p<0.05$). Thereby tumour growth inhibition was increased by 82.6% for mice injected with 161Tb-DOTA-chCE7 compared to the 177Lu-DOTA-chCE7 therapy group. High comparable tumour uptakes for both radioimmunoconjugates (RICs) of 37.2% ± 1.0% (177Lu-DOTA-chCE7) and 43.6% ± 3.4% (161Tb-DOTA-chCE7) were shown 144 h post RIC injection ($p>0.05$). Our study is the first to show that 161Tb-RIT is more effective in an ovarian cancer model under equitoxic conditions in comparison to 177Lu-RIT [3]. Therefore, 161Tb is potentially the better candidate for RIT with internalising antibodies.

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Function of Dna2 in eukaryotic DNA replication

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Dna2 is a nuclease-helicase involved in several pathways of eukaryotic DNA metabolism with an essential function in DNA replication. The expression of Dna2 was increased in human cancers and negatively correlated with disease outcome, while depletion of Dna2 lowered proliferation and tumorigenicity in mice. However, the role of Dna2 in replication remains poorly defined and mechanistic insights in Dna2 function could help to understand its tumorigenic potential. Dna2 is known to be involved in replication during lagging strand DNA synthesis that occurs in stretches termed Okazaki fragments. Before adjacent fragments are ligated, any flaps resulting from DNA displacement of the 5\' end of the Okazaki fragment must be cleaved. Previously, Dna2 was implicated to function upstream of flap-endonuclease 1 (Fen1) in the processing of long flaps bound by the replication-protein A (RPA). Here we show that fully active yeast Dna2 protein (1) efficiently cleaves DNA near the flap base, and subsequent nucleolytic activity of Fen1 is not required. When coupled with replication, cleavage of long flaps by Dna2 promotes nearly complete Okazaki fragment maturation. We show that also Dna2, like Fen1, interacts with PCNA. We propose a model where Dna2 alone is responsible for cleaving long flaps, while Fen1 or Exo1 cleaves short flaps. Our results argue that Dna2 can function in a separate, Fen1-independent pathway, and may explain why dna2Δ is lethal, while fen1Δ is not (2). Additionally, we could show in collaboration that under conditions of replication stress Dna2 was capable of processing of stalled replication forks after fork reversal (3).

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ATP-dependent formation of viral ribonucleoprotein particles in oncogenic herpesvirus infection: a target for small molecule inhibitors?

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncovirus associated with multiple malignancies, including Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease. The virus replicates in the nucleus of the host cell and requires cellular factors to export viral mRNAs from the nucleus, to allow translation of viral proteins in the cytoplasm. However, while mammalian mRNA export is coupled to splicing, the majority of KSHV mRNAs are intronless, prompting the virus to circumvent this step. KSHV therefore encodes ORF57, a protein which interacts with the human transcription/export (hTREX) complex. This allows for nuclear export of viral mRNAs and subsequent translation of viral proteins. In this study we present a novel mechanism for specific disruption of the ORF57/hTREX interaction and subsequent inhibition of vi rus lytic replication. This is a key finding, as lytic replication is an essential factor in KSHV mediated tumourigenesis. For the first time, we show ATP-cycle dependent remodelling of the hTREX complex, which affects the ability of ORF57 to recruit the endogenous proteins. Following this, we target a cellular ATPase, which is an essential hTREX component, using small molecule inhibitors identified by virtual high-throughput screening. Strikingly, hit compounds were able to impair ATP hydrolysis and thereby formation of the viral ribonucleoprotein particle, while allowing endogenous hTREX formation. As a consequence, viral mRNA export is ef-

ficiently disrupted, while endogenous mRNA export and cell viability are not affected. Together, we present compounds that are able to prevent KSHV late protein expression, reduce viral load and disrupt lytic replication, with limited cytotoxicity.

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Impact of pharmacological inhibition of CSF-1R on the formation of metastases

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Whereas localized tumours are clinically manageable, most patients succumb to metastatic disease. Tumour-associated macrophages (TAMs) have been widely associated with a tumour-promoting environment, induction of immune tolerance to cancer cells, increased rate of metastasis formation and unfavourable prognosis. One of the pathways crucial for TAMs survival and their differentiation from precursor cells is orchestrated by colony stimulating factor 1 (CSF-1). We have used a small molecule inhibitor of its receptor CSF-1R (CSF1-Ri) in a murine model of spontaneous metastases from subcutaneously injected tumours. Treatment with the CSF-1Ri as an adjuvant to surgery (starting the treatment from the day of surgery and continuing until the endpoint) did not reduce the rate of metastasis formation. This suggests that CSF-1R-dependent myeloid cells don't play an important role for the outgrowth of already-formed metastases. When the treatment was started on the same day as injection of tumour cells, the tumours grew significantly faster. Analysis of immune infiltrate of the tumours revealed decreased numbers of F4/80+ MHC+ CD11c+ myeloid cells and an increase of the ratio of regulatory T cells to CD8+ T cells. These data suggest a role of CSF-1R-dependent myeloid cells in mediating tumour protection against early tumours, probably through interference with priming of adaptive, tumour-specific immunity. We are currently dissecting the exact impact of CSF-1R-dependendt myeloid cells on different aspects of tumour progression in order to develop a rational schedule for the treatment of cancer with CSF-1Ri.

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The impact of Tousled-like kinases on development and disease

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The plasticity of chromatin structure and the maintenance of genomic stability are crucial for cellular and developmental processes, such as the production of the blood components [1]. The Tousled-like kinases (TLK1, TLK2) have been implicated in chromatin assembly, transcriptional regulation and genome stability, as they interact with and regulate the histone chaperone ASF1[2]. To address their relative roles, we generated mice lacking either TLK1 or TLK2. While TLK1 deficient mice do not show any overt defects, TLK2 deficient mice perish during embryogenesis due to transcriptional defects, impaired trophoblast differentiation and placental failure by day 15.5 of embryo development (e15.5). Therefore, TLK2 is uniquely required for embryonic development due to a cell type specific role [3]. To further address the individual and combined roles of the TLKs in mammalian development, we have established a conditional allele for TLK1 and TLK2 to allow tissue specific deletion. Using CD19-Cre mice, we depleted TLK2 in the B-cell lineage and assessed the impact on B-cell development in aging mice. TLK2 deficient B-cell precursors in the bone marrow accumulate at the transition from large Pre-B to small Pre-B cells. This is accompanied by an expansion of the splenic lymphocyte population and loss of splenic architecture. Based on this preliminary data, we hypothesize that TLK2 activity is required at specific stages of B-cell development, likely due to its regulation of chromatin structure and gene expression, a possibility we are currently testing. Ongoing work to establish the relative influence of TLK1 and TLK2 on lymphocyte development will be presented.

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MiRNA based classification of breast cancer subtypes

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The classification of breast cancer by immunohistochemical parameters into distinct molecular subtypes creating six specific subtypes (Luminal A, Luminal B, Luminal Her2, Her2-enriched, Basal-like and Triple-negative) has become a standard in clinical routine. Only four parameters (estrogen receptor, progesterone receptor, Her2/neu receptor and Ki67) are determined by immunohistochemical staining. However, this characterization system has limitations. MiRNAs are known regulators in cancer progression. Our study investigated breast cancer subtype specific miRNA expression patterns and their robustness as prognostic and predictive biomarkers in the clinical setting. Sixteen subtype-specific and prognostically relevant miRNAs were identified by a comprehensive Pubmed query. After clinico-pathological review, serial sliced tissue samples from all subtypes were micro-dissected. MiRNA was quantified using PCR. MiRNA expression levels of the distinct 16 specific and 2 control miRNAs were integrated into an artificial neuronal network as an innovative, multiparametric, biostatistical tool in this setting for statistical analysis. In 90 of 94 cases the miRNA based profile of distinct 16 miRNAs predicted the expected intrinsic subtypes by applying the artificial neuronal network. Specificity of subtype determination ranged from 100% (Basal-like) to 85% (Triple-negative) and sensitivity from 100% (Basal-like) to 99% (Triple-negative), respectively. Further analyses were performed for prediction of lymph node involvement. Sensitivity and specificity for lymph node involvement in clinically negative patients cN0 was calculated with 68% and 72%, respectively. We consider that this study provides first insight into the prognostic and predictive impact of molecular miRNA expression patterns on the intrinsic subtype and clinico-pathological parameters.

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Biochemical analysis of human DNA2

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Impaired DNA helicase activity can lead to genomic instability that is a hallmark of cancer. Mutations in either the helicase- or nuclease-domain of human Dna2 enzyme were found in ovarian cancer tumors, probably triggering tumorigenesis at early stages(1). Furthermore, cancer cells must deal with replication stress-induced lesions like DNA double strand breaks (DSBs). The expression of Dna2 is significantly increased in human cancers and inversely correlates with the disease outcome(2). One pathway to repair DSBs is homologous recombination that needs extensive DNA end resection generating 3\' single stranded (ss) DNA overhangs. Dna2 was shown to promote long-range DNA resection(3). Therefore it is assumed that Dna2 can reduce replication stress in tumors and provide cancer cells with a survival advantage under such conditions. Inhibitors targeting helicases/nucleases affecting DSB repair have a high potential as anti-cancer drugs. However, the biochemical characterization of human Dna2 is not complete yet and also the essential function of the protein in vivo is not clearly defined so far. We show that Dna2 is a potent helicase that can unwind up to 2.7 kbp of DNA in a RPA- and ATP-dependent manner. However, the helicase activity is masked by the enzyme\'s nuclease activity similarly as shown for yeast Dna2 before. We demonstrate that the helicase activity of DNA2 speeds up the degradation of ssDNA by the nuclease domain of the enzyme, most likely through a translocase function.

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ESE3/EHF is involved in androgen receptor signaling and disease progression in prostate cancer

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Prostate cancer is a leading cause of cancer-related deaths. Androgen-deprivation therapy (ADT) is a standard of care treatment for prostate cancer. However, despite the initial efficacy of ADT, castration-resistant disease frequently occurs and the underlying mechanisms are not fully understood. ETS transcription factors have emerged as important elements in the pathogenesis of prostate cancer. Our previous studies indicated that the ETS factor ESE3/EHF is a tumor suppressor and its loss results in activation of important oncogenic pathways in prostate tumors. In this study we investigated the impact of the loss of ESE3/EHF on the phenotype of androgen receptor (AR) positive prostate cancer cells and their response to anti-androgen compounds. We knocked down ESE3/EHF in LNCaP cells, an androgen positive cell line. In vitro assays (soft agar, sphere forming, clonogenic and wound healing assays) were carried out to compare parental and ESE3/EHF knockdown LNCaP cells. Furthermore, the response to the anti-androgens bicalutamide (Casodex) and enzalutamide (MDV) was evaluated. Downregulation of ESE3/EHF in LNCaP cells promoted anchorage-independent growth, prostatosphere formation and migration. ESE3/EHF knockdown LNCaP cells exhibited increased tumorigenicity and formed larger tumors compared to control cells in NOD/SCID mice. Knockdown of ESE3/EHF also reduced the response to enzalutamide and bicalutamide compared to control cells. Collectively, these data indicate a role of ESE3/EHF downregulation in tumor progression and resistance to ADT. These findings may have important therapeutic impact for the management of prostate tumors.

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The epigenetic modifier EZH2 controls melanoma growth and metastasis through silencing of distinct tumour suppressors

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Activity of the epigenetic modifier EZH2, a methyltransferase that represses gene expression through trimethylation of H3K27, has been associated with clinical outcome and formation of several cancers. However, evidence for a functional role of EZH2 in tumorigenesis in vivo remains poor, particularly for solid cancers. Here we reveal central roles of EZH2 in promoting growth and metastasis of cutaneous melanoma. In a transgenic mouse model of melanoma(1), conditional Ezh2 ablation counteracted growth of cutaneous tumors without affecting normal melanocyte biology. Importantly, whether Ezh2 was ablated before or after the onset of skin melanomagenesis, emergence of distant metastases was completely prevented. Likewise, treatment of melanoma-bearing mice with the preclinical EZH2 inhibitor GSK503 virtually abolished metastases formation, resulting in prolonged survival. In agreement with the in v ivo phenotypes, EZH2 inactivation in human melanoma cells impaired proliferation, but most apparently prevented EMT and invasion in Boyden chambers. Furthermore, interference with EZH2 activity in these cells allowed the identification of novel EZH2 target genes (ETGs) using transcriptome and ChIP assays. In a cohort of melanoma patients, high expression of these ETGs correlated with prolonged survival. In contrast, silencing of ETGs promoted either melanoma cell proliferation or metastases in vivo, establishing these ETGs as a functionally diverse set of tumor sup-

pressors. Thus, EZH2 acts as a central node in driving melanoma growth and metastatic progression through dynamic repression of distinct tumor suppressors, which makes EZH2 a highly promising target for novel melanoma therapies(2).

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The DNA damage-induced phosphoproteome is modulated by inhibition of the receptor tyrosine kinase MET

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Ionizing radiation (IR), is frequently used in the treatment of a variety of malignant tumors of different origins and stages. In recent years, numerous studies have demonstrated that interfering with signaling via growth factor receptor tyrosine kinases (RTKs), such as the RTK for hepatocyte growth factor, known as MET, can increase the sensitivity of certain tumors to IR. In this study, we have aimed to explore how the cellular response to ionizing radiation is modulated by MET inhibition. We have conducted a phosphoproteomics survey study to explore the cellular phosphoproteome following exposure of MET-addicted cancer cells to METi alone and in combination with IR. Analysis of the survey data has identified more than 300 phosphopeptides which have changed in one experimental condition or more. Several of these phosphorylation changes have been confirmed and further investigated by targeted proteomics. These results have pointed to a sub-network of the DNA damage response that is activated in MET-addicted cancer cells upon DNA damage and MET inhibition, and could be responsible for synergism between these two modalities. We hope that this work will aid in understanding how treatment with such combinations could improve the clinical outcomes of patients.

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Base excision repair initiation in the context of chromatin

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Several thousand harmful and mutagenic DNA base lesions are generated within a single mammalian cell every day. Base excision repair (BER) is an essential pathway for removal of damaged DNA bases that is initiated by one of eleven human DNA glycosylases. Though mammalian cells need to repair damaged DNA in the context of chromatin, little is known about how chromatin structure affects DNA glycosylases. At present it is further unknown which chromatin-modifying enzymes specifically facilitate BER initiated by particular DNA glycosylases. Here we show that the activity of a particular DNA glycosylase is inhibited in the presence of nucleosomes compared to naked DNA. We identified a histone acetyltransferase (HAT) as a novel interaction partner of the DNA glycosylase. The interaction between the DNA glycosylase and HAT is direct, as well as DNA and RNA independent. Interestingly, cells lacking the functional HAT show increases in level of DNA base lesions and are more sensitive to treatment with DNA damaging agents. Taken together, we identify for the first time the association of a specific HAT with components of BER and the potential consequences of this interaction on BER initiation will be discussed. This work is supported by SNSF.

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Insulin receptor is upregulated on tumor-associated vessels by hypoxia and facilitates metastasis of bladder cancer

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Tumor–associated blood vessels are morphologically and functionally abnormal. Identification of differentially expressed molecules on tumor-associated vessels compared to vessels of normal tissue can lead to identification of novel biomarkers and therapeutic targets. We compared the transcriptome of isolated blood vascular endothelium from human invasive bladder cancer and from normal bladder tissue and identified upregulation of insulin receptor (INSR). We found particularly high expression of INSR on vessels of bladder cancer and in a wide range of solid cancers. INSR expression on tumor vessels strongly correlated with reduced progression-free survival and reduced overall survival in invasive bladder cancer and predicted a shorter recurrence-free survival in non-invasive bladder cancers. Using cultured blood vascular endothelial cells (BECs), we found that hypoxia upregulates INSR via binding of HIF1A to a putative enhancer in the INSR regulatory sequence. Additionally, we observed hypoxia in bladder cancer and in tumor-associated blood vascular endothelial cells. We found that the expression of INSR isoform-A predominates over expression of INSR isoform-B. INSR-mediated migration of BECs in vitro by increased NO production via stimulating endothelial nitric oxide synthase (eNOS). Deletion of INSR on endothelial cells (ECs) in tumor bearing mice (B16F10 and MB49), down-regulated expression of VEGF-A and eNOS in ECs, leading to reduction of hypoxia in tumors, possibly as a result of decreased permeability and better oxygen transport over the EC barrier, leading to reduced metastasis. Altogether, INSR might serve as a useful biomarker to monitor disease progression and selective inhibition of INSR-A could offer new therapeutic opportunities for inhibiting cancer metastasis.

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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). Notably, elevated numbers of intratumoral effector T-cells significantly correlates with a better survival of patients diagnosed with GB. In our lab, we use the GL-261 glioma experimental model to study the consequences of interleukin-12 (IL-12) treatment on the tumor microenvironment from a qualitative and quantitative aspect. IL-12, a heterodimeric pro-inflammatory cytokine, polarizes naïve helper T-cells into a Th1 phenotype and stimulates cytotoxic T-cells, natural killer (NK) T-cells and conventional NK cells. Intratumoral IL-12 has been investigated in different experimental brain tumor immunotherapy models, leading to long-term antitumor immunity(2). Our main hypothesis is that local IL-12 leads to reversal of the suppressive tumor microenvironment into a pro-inflammatory microenvironment with increased antigen presentation supportive of an adaptive immune response. In our model, IL-12 induced a strong infiltration of activated CD4+ and CD8+ T-cells and subsequent elimination of tumor cells. According to our data, perforin-dependent T-cell cytotoxicity is the major effector mechanism of IL-12-mediated glioma rejection(3). However, while the ultimate effectors are well established, the contribution of IL-12 in triggering this response remains elusive. We aim to identify the responsible antigen presenting cell subset as well as elucidating the site of antigen presentation leading to T-cell activation. 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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MAP4K4 mediates c-Met-dependent dissemination of Shh Medulloblastoma through targeting of actin dynamics

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Medulloblastoma (MB), the most common malignant pediatric brain tumor, currently comprises four molecularly different tumor entities named Wingless (Wnt), Sonic hedgehog (Shh), Group 3 and Group 4 MB. MB is characterized by a high risk of leptomeningeal dissemination, which accounts for the poor clinical outcomes of MB patients. Molecular mechanisms leading to dissemination are poorly understood and no targeted anti-metastatic therapies exist. The activation of the oncogenic c-Met signaling cascade is associated with 80% of Shh and 25% of Group 3 tumors and correlates with dissemination, poor prognosis and frequent recurrence induced by currently-unknown molecular mechanisms. We report functional, molecular and morphological alterations induced by HGF-c-Met signaling in a cell-based model of Shh MB. We demonstrate that HGF-induced c-Met activation promotes motility of single cells through the activation of the Ser/Thr c-jun N-terminal kinase JNK. Using a novel collagen 3D dissemination assay, we identified the Ser/Thr Kinase MAP4K4 as novel player relevant to Shh MB dissemination. We show that MAP4K4 function is necessary both for constitutive as well as c-Met-induced motile behavior of Shh MB cells. We ascribe this function of MAP4K4 to its control of F-actin cytoskeleton dynamics in structures required for migration and invasion, revealing a novel aspect of growth factor-induced signaling essential for MB dissemination. Combined, these results demonstrate that c-Met promoted dissemination of Shh MB depends on MAP4K4 and suggest that this kinase could present a promising, subgroup-specific target to be evaluated for treating growth factor-induced dissemination of high-risk Shh MB.

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Aberrant STAT3-ARF signaling targets distinct subgroups of lethal prostate cancer

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Prostate cancer (PCa) is one of the most prevalent forms of cancer and the second most common cause of male cancer mortality. While various clinical and biochemical prognostic factors for survival and progression of PCa have been proposed, the stratification of high low-risk PCa patients who will suffer from disease recurrence remains limited. We investigated the role of IL-6/Stat3 signaling by combining the Pten-deficient PCa mouse model with direct translation relevance for men with advanced PCa. We generated transgenic mice harboring Pb-Cre4 and conditional knockout alleles of Pten and/or Stat3 (hereafter referred to as Ptenpc-/- Stat3pc-/-). We also crossed Ptenpc-/- mice with IL-6-/- mice to demonstrate the effect of IL-6 inactivation on PCa progression. Here we show that genetic inactivation of Stat3 dramatically promotes cancer progression in a Pten-deficient PCa mouse model leading to metastasis. Mechanistically, we identified p19ARF as a novel direct Stat3 target and demonstrate that loss of Stat3 signaling disrupts the ARF-Mdm2-p53 tumor suppressor pathway bypassing senescence. Consistent with these findings, loss of STAT3 and p14ARF expression in patient tumors correlates with increased risk of disease recurrence and metastatic PCa. Strikingly, we also identified STAT3 mutations in 2.8% of primary PCa and frequent STAT3 and/or p14ARF deletions (21%) in metastatic PCa. Taken together, we have discovered novel markers to stratify high and low-risk PCa patients and revealed that STAT3 and p14ARF abrogations may define new molecular subtype of lethal PCa with potentially important therapeutic implications.

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Development of different tumour-associated autoantibody detection platforms - comparison of the technical performance

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Autoantibodies against tumour-associated antigens (TAAs) can be found early during cancer development and therefore may represent valuable diagnostic biomarkers. However, the immune system generates antibody response against a wide variety of TAAs, and the cancer patients' autoantibody repertoire partially overlaps with that induced by inflammation and/or autoimmune processes. To identify tumour-associated autoantibodies with most promising diagnostic value, there is a need for high-throughput methods that enable the analysis of a wide repertoire of different cancer antigens in large cohorts of test samples. In this study, we developed two different tumour-associated autoantibody detection platforms, Luminex® microbead- and microarray-based assays, and compared their technical performance, including technical sensitivity, dynamic range, variation coefficients, as well as comparison of different microarray slide surface chemistries (epoxyde, aldehyde, hydrogel, nickel and nitrocellulose). In total, 67 TAAs with diagnostic value identified within our previous studies were expressed recombinantly as His-HaloTag fusion proteins and tested with selected serum samples of known reactivity by using the developed assays. The results showed that the least variation between replicates and experiments was on aldehyde and nickel microarrays - 4.5-5.3% and 7-7.8%, while Luminex® microbeads had 7% and 8%, respectively. Dynamic range of anti-HaloTag® antibody detection retaining linear correlation between antibody dilution and signal intensity was 1:500-1:1250 for all platforms. Technical sensitivity was highest for the hydrogel slides (yet with most variable spot morphology), followed by aldehyde and nickel microarrays, the lowest - for Luminex® microbeads. Taking the data together, nickel microarrays were determined as optimal due to best combination of all tested parameters.

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Molecular portrait of the fatal TCF3-HLF leukemia

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TCF3-HLF fusion gene-positive acute lymphoblastic leukemia (ALL) is currently not curable. Integration of multi-omic sequencing data from primary and matched xenograft samples derived from TCF3-HLF-positive and treatment-responsive TCF3-PBX1-positive ALL patients revealed subtype-specific mutation patterns. In TCF3-HLF-positive ALL, recurrent intragenic deletions of the lymphoid transcription factor gene PAX5 or somatic mutations in the non-translocated allele of TCF3 (acting upstream of PAX5) were common and frequently in conjunction with RAS pathway aberrations. Despite a lymphoid-committed cell of origin, the transcriptional signature of TCF3-HLF-positive ALL was enriched for stem cell, mesenchymal, and myeloid features, consistent with reprogramming towards a hybrid, more drug-resistant hematopoietic state. TCF3-HLF-positive ALL revealed a distinct drug-response profile with resistance to some agents commonly used for its treatment, but sensitivity towards glucocorticoids and other drugs in clinical development. Striking on-target sensitivity was achieved with the BCL2-specific inhibitor ABT-199 indicating a strict BCL2-dependency. Our integrated approach has discovered a potential alternative treatment for a fatal disease.

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Molecular and cellular mechanism of metastatic cancer cell dissemination into the brain

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Brain metastasis is a fatal complication for cancer patients caused by the dissemination of metastatic cancer cells (MCCs) into the central nervous system (CNS). Because the CNS is devoid of lymphatic vessels the only route of MCC dissemination into the brain is the blood vasculature tightly sealed by blood brain barrier (BBB). The BBB is formed by highly specialized endothelial cells of the parenchymal CNS microvessels and protects the vulnerable physiology of the brain from the changeable milieu in the blood. While extravasation of effector/memory T cells (TEM cells) across the BBB has been subject of intense research [1], the mechanism of MCC extravasation across the BBB remains obscure. Some reports describe arrest of MCCs simply as the result of their large size relative to the capillary vessel while others describe expression of trafficking molecules known to be involved in TEM cell extravasation such as α4-integrin. In this study we translated our longstanding experience from studying the mechanism of TEM cell extravasation across the BBB [2, 3] into analysis of the molecular and cellular mechanism of MCC extravasation across the BBB. In vitro live cell imaging under physiological flow condition reveals fundamental differences between the extravasation of MCC and TEM cell extravasation across the BBB. Moreover, metastatic breast carcinoma or melanoma cells use distinct molecular mechanism for adhesion to the BBB under flow. Our preliminary observations support the idea of the healthy BBB as a protective shielding against MCC dissemination into the brain.

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Genetic and phenotypic diversity of BRAF mutations in lung cancer

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BRAF mutations have been identified as a potential therapeutic target in a variety of human cancers. Our aim was to determine clinical, morphologic and molecular characteristics of BRAF mutated lung cancer samples. Consecutive lung cancers (2,529 cases) were tested for BRAF mutations as part of a comprehensive genetic characterization. Immunohisto-chemical staining for the V600E mutation was carried out in a subset of cases. Fifty six tumors harbored a BRAF mutation, among them 54 (96.4%) adenocarcinomas, one (1.8%) squamous and one sarcomatoid carcinoma (1.8%). We identified five different mutational subtypes in exon 11, including one newly described in this report. Among thirteen different mutational subtypes in exon 15, five are here reported for the first time. Hence, having evaluated the largest series of unselected lung cancers thusfar assessed to our knowledge, we could determine the frequency of BRAF mutations at 2.2% and show that BRAF mutations occur predominantly in solid and acinar subtypes of adenocarcinomas. The spectrum of BRAF mutations in pulmonary carcinomas is broader than previously thought with only 39.3% accounting for the V600E subtype (1% of all cancers).

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Sensitization of cancer cells to cisplatin

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Platinum compounds like cisplatin and carboplatin are DNA-crosslinking agents widely used in cancer chemotherapy that induce excellent initial responses against a variety of solid tumors. However, chemotherapy-induced resistance of cancer cells to platinum-based agents poses a major clinical problem. Until now, no pharmacologic approach has successfully overcome cisplatin resistance in cancer treatment. The cytotoxicity of cisplatin is mediated by the introduction of DNA intrastrand and interstrand crosslinks that inhibit transcription and DNA replication. Several previous studies indicate that protein turnover regulated by multiple mono- or polyubiquitination reactions plays a pivotal role in the cellular responses to platinum-induced DNA crosslinks. Therefore, we are currently testing whether small-molecule inhibitors of the protein ubiquitination cascade may sensitize cancer cells to the action of platinum-based chemotherapeutics. Initial results indicate that PYR-41, an inhibitor of the ubiquitin-activating enzyme E1, enhances the cytotoxicity of cisplatin towards HeLa cells. At a non-toxic concentration of 20 μM, PYR-41 reduces the IC50 of cisplatin from 7.1 μM to 2.5 μM. The effect of PYR-41 on cisplatin cytotoxicity is greater than that observed by i) down regulation of the CUL4A/CUL4B ubiquitin ligases involved in protein ubiquitination, ii) down regulation of XPA or iii) down regulation of the XPF-ERCC1 and XPG endonucleases involved in DNA crosslink repair. However, inhibition of the ubiquitin-dependent p97 segregase by either NMS-873 or DBeQ did not have any effects on the sensitivity of HeLa cells towards cisplatin. Surprisingly, we also found that the cisplatin-resistant ovarian cancer cell line CP70 is hypersensitive to treatment with the ubiquitin-activating enzyme E1 inhibitor PYR-41. These findings provide evidence that ubiquitination plays an important role in the processing of cisplatin-induced DNA damage, but the underlying mechanisms need to be further elucidated and further screening is necessary to identify the most susceptible target in both cisplatin-sensitive and cisplatin-resistant cancer cells.

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BAZ2A (TIP5) is involved in epigenetic alterations in prostate cancer and its overexpression predicts disease recurrence

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Prostate cancer is the most common non-cutaneous malignancy in men. Prediction of clinical behavior remains challenging using the currently available histopathological and biochemical (prostate-specific antigen; PSA) markers; thus, novel molecular-based approaches will likely improve prognostic accuracy in this disease. Cancer evolution is driven by a combination of genetic and epigenetic abnormalities; the crucial role of epigenetic gene regulation is indicated by the high frequency of mutations in key epigenetic regulators. As prostate cancer is characterized by a low frequency of somatic mutations, disruption of epigenetic pathways is likely to have an important role in the disease. Here we show that the gene encoding BAZ2A (TIP5), a factor previously implicated in epigenetic silencing of rRNA genes, is overexpressed in prostate cancer and is paradoxically involved in maintaining prostate cancer cell growth, a feature specific to cancer cells. Moreover, BAZ2A is required for migration, invasion and self-renewal of metastatic prostate cancer cells. BAZ2A cooperates with the histone H3K27 methyltransferase EZH2 to establish epigenetic silencing of genes frequently repressed in metastatic tumors. BAZ2A overexpression is tightly associated with a prostate cancer subtype displaying a CpG island methylator phenotype (CIMP). Finally, high BAZ2A levels serve as an independent predictor of biochemical recurrence in a cohort of 7,682 individuals with prostate cancer. Our results demonstrate that BAZ2A is a new biomarker that distinguishes aggressive disease and suggest a role for BAZ2A in the establishment of epigenetic alterations in aggressive prostate cancer.

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CDCP1 interacts with HER2 to enhance HER2-driven tumorigenesis and promote trastuzumab resistance

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More than half HER2-overexpressing breast cancer patients do not respond to trastuzumab-based therapies. Understanding the molecular pathways that contribute to the aggressive behavior of HER2-positive breast cancers may help to develop novel therapeutic strategies. Here, we found that CUP-domain-containing-protein 1 (CDCP1) and HER2 are frequently co-overexpressed in metastatic breast tumors, and this is associated with a poorer patient prognosis. Mechanistically, we demonstrated that CDCP1 interacts with HER2 through its intracellular-portion, thereby increasing HER2 interaction with the non-receptor tyrosine kinase c-SRC (SRC). As a consequence, SRC phosphorylates HER2 thereby promoting HER2 signaling. On this line overexpression of CDCP1 resulted in activation of SRC/HER2 axis leading to trastuzumab resistance in vivo. Taken together, our findings suggest that CDCP1 is a novel modulator of HER2 signalling, identifying a novel biomarker for the stratification of trastuzumab resistance breast cancer patients. Our findings also provide the rationale for the therapeutic targeting of CDCP1 in HER2-positive breast cancer patients.

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Phosphoenolpyruvate is a metabolic checkpoint controlling Ca2+-NFAT signaling and anti-tumor T cell responses

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Activated T cells engage aerobic glycolysis and anabolic metabolism for growth, proliferation and effector functions. We propose that the glucose-poor tumor microenvironment limits aerobic glycolysis in tumor-infiltrating T cells, which suppresses TH1 tumoricidal effector functions. We discovered a new role for the glycolytic metabolite phosphoenolpyruvate (PEP) in sustaining T cell receptor-mediated Ca2+-NFAT signaling and effector functions by repressing sarco/endoplasmic reticulum Ca2+-ATPase activity. Tumor-specific CD4 T cells could be metabolically reprogrammed by increasing PEP production through overexpression of phosphoenolpyruvate carboxykinase 1 (PCK1), which bolstered TH1 effector functions. Moreover, PCK1-overexpressing TH1 cells restricted tumor growth and prolonged the survival of melanoma-bearing mice. This study uncovers new metabolic checkpoints for T cell activity and demonstrates that metabolic reprogramming of tumor-

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Reviewing 30 years of Raf kinase - predictions for anti-cancer therapy

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Cancer research was stimulated decades ago by analysis of retroviral oncogenes. These genes opened the understanding for human cancer. Many of them influence growth control, characterized as a hallmark of cancer. Oncogenes include Ras, ErbB2, Fos, Jun, Ras-Myc, receptor tyrosine kinases, EBNA; tumor suppressors include p53 or Rb. Many have been targeted to fight cancer with limited therapeutic success. The oncogene Mil/Raf was described 30 years ago in two retroviruses as an essential signal transducer with unexpected serine-threonine kinase activity (1). Later this kinase was shown to be a driving force in many human cancers, especially if partially activated by certain prominent mutations. The Raf kinase was shown to play an unexpected dual role, designated as Janus head, in that Raf is not only activated in cancer cells, but a strong Raf activity can also lead to cell-cycle arrest and differentiaion. The Raf-kinase activity is regulated by phosphorylation by the AKT-kinase in a cross-talk with the PI3 kinase pathway (2). Such dual mechanism subsequently opened the understanding for duality of other oncogenes, e.g. Fos and Ras. Several novel Raf kinase inhibitors showed transient efficiencies or even opposite effects - to be expected for a Raf kinase in a differentiation setting. We furthermore demonstrated a negative feedback loop with "upstream signalling" from Raf-MEK to receptor kinases, which were intensified by inhibtors. Dual therapies including MEK inhibitors and/or blockage of receptors overcame these problems - predictable by the models described (3), suggesting that they should be considered and that non-mutated Raf could also play a role in cancer.

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Mouse embryonic stem cells complete genome duplication while the next replication round is ongoing

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Embryonic stem cells (ESCs) represent a transient biological state, where pluripotency is coupled with fast proliferation. This early stage of embryogenesis is reportedly associated with activation of the DNA damage response (DDR), but its molecular determinants and physiological value have remained elusive. Here we show, in cultured ESCs and in mouse pre-implantation embryos, that ATR-dependent H2AX phosphorylation (γH2AX) is associated with chromatin loading of the single-stranded (ss)DNA-binding proteins RPA and RAD51. Accordingly, single-molecule analysis of replication intermediates in unperturbed ESCs reveals massive ssDNA gap accumulation, reduced replication fork speed and frequent fork reversal. Genetic inactivation of fork reversal by PARP inhibition leads to extensive chromosomal breakage. All

these marks of replication stress in ESCs are rapidly lost upon induction of differentiation. Delaying the G1/S transition in ESCs allows formation of 53BP1 nuclear bodies and alleviates the observed DDR, suppressing ssDNA accumulation, fork slowing and reversal in the following S phase. Our data shed light on unexpected peculiarities of chromosomal replication in ESCs. We propose that ESCs perform genome duplication while still completing the previous replication round and maintain genome integrity by effective replication-coupled repair and fork protection systems.

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Inhibition of autophagy sensitizes malignant pleural mesothelioma cells to dual PI3K/mTOR inhibitors

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Background: PI3K/mTOR pathway is deregulated in MPM. The aim of this study is to explore the therapeutic potential of two dual PI3K/mTOR inhibitors and to identify and target resistance mechanisms in order to induce cell death.

Methods: Cell growth inhibition upon treatment of NVP-BEZ235 or GDC0980 was assessed by MTT assay in 19 MPM cell lines. Down-regulation of PI3K/mTOR signaling was assessed determining AKT, S6 and 4E-BP1 phosphorylation. Two resistant and sensitive cell lines were selected. Autophagy induction was monitored by WB using LC3 and p62 autophagy markers. Inhibition of cell cycle and induction of cell death in combination treatment with chloroquine and NVP-BEZ235 or GDC0980 was determined using Annexin V and PI by FACS.

Results: Sensitive and resistant cell lines were selected and confirmed in 3D systems. Sensitive cell lines showed G1 cell cycle arrest with both drugs but no cell death. All cell lines showed an increase of autophagy after drug treatment. The inhibition of autophagy by chloroquine in combination with NVP-BEZ235 or GDC0980 showed a stronger effect in cell growth inhibition in both resistant and sensitive cell lines, inducing significant caspase-independent cell death in 2D and more pronounced 3D systems.

Conclusion: Autophagy appears to be one of the main mechanisms of cell death resistance against dual PI3K/mTOR inhibitors in MPM. Since this therapeutic option is under investigation in clinical trials, these results may help interpreting their outcome, and suggest ways for intervention.

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Elucidating the role of MMS22L-TONSL heterodimer in homologous recombination

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MMS22L (Mms22-like) forms a complex with another human protein, TONSL (Tonsoku-like). MMS22L-TONSL complex localizes at sites of damaged DNA upon laser microirradiation. Depletion of MMS22L or TONSL results in the hypersensitivity to a variety of DNA-damaging drugs that interfere with DNA replication. Furthermore, depletion of MMS22L or TONSL resulted in persistent RPA foci and decreased formation of RAD51 foci. These data suggest that this protein complex might play a role in the initiation of Homologous recombination (HR)-dependent repair (1). The aim of this project is to perform biochemical analysis to investigate the potential role of MMS22L-TONSL in HR, in particular as a recombination mediator. Recombination mediators, such as BRCA2, help load RAD51 on RPA-coated ssDNA. RAD51 then performs search for homologous DNA duplex, which leads to HR repair. First, we purified MMS22L-TONSL complex from Sf9 insect cell expression system. The complex purifies as heterodimer. Then we tested its ability to bind various DNA substrates. We found that MMS22L-TONSL complex binds long stretches of DNA, suggesting that DNA might be bound cooperatively. Next we tested direct protein-protein interactions between MMS22L-TONSL or MMS22L and RAD51. Strikingly, RAD51 binds both MMS22L-TONSL and MMS22L, suggesting that MMS22L is the interacting subunit. MMS22L-TONSL also stimulates RAD51 strand exchange activity and lowers binding affinity of RAD51 to dsDNA. We will continue the analysis of biochemical behavior of MMS22L-TONSL in HR in the context of stalled replication forks, as well as the interplay of MMS22L-TONSL with already known mediators, including RAD51 paralogues and BRCA2 (2, 3).

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Epigenetic Therapy a Good BET for Glioblastoma? A systems biology approach to identify a rational combination therapy

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The dependency of cancer cells on the interplay between aberrant changes of the epigenetic landscape and genetic alterations is rapidly emerging. Understanding chromatin control of gene expression, such as the relationships between histone modifications, including acetylation and DNA methylation, may hold the key to novel cancer treatment strategies. We aim to address the question of functional contribution of the epigenetic landscape to malignant glioblastoma (GBM) cell behaviour in order to identify novel drug combination strategies for personalized therapy. Our research is based on the clinical development of potent inhibitors of chromatin "readers", namely bromodomain and extra-terminal (BET) proteins that seem to be promising targets in hematopoietic malignancies, and potentially also in solid tumours including gliomas. The BET inhibitor compound JQ1 is used as a tool in our in vitro models. Our results show moderate sensitivity of glioma sphere lines to JQ1. The subsequent analysis suggests that GBM cells do not enter apoptosis, but undergo G1-arrest and acquire a senescence-like phenotype, as determined by presence of SA-beta-GAL-activity. Finally, recent results suggest the presence of a common gene expression signature that represents differential expression between cells under BET inhibitor exposure and control conditions. Further investigation will focus at disturbing the system by using BET inhibitors, identifying disturbed pathways which we hypothesize should be informative for potential vulnerabilities that may be targeted with a second drug to be used in combination with potent BET inhibition.

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Detection and Quantification of Extracellular MicroRNAs in Medulloblastoma

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Medulloblastomas (MB) are the most common brain tumors in children. Medulloblastomas exhibit aberrant expression of intracellular microRNAs compared with normal cerebellum. In recent years, microRNAs have been found in a wide spectrum of human body fluids as extracellular circulating nuclease-resistant entities. Hence the diagnostic and prognostic potential of extracellular microRNAs has been demonstrated in preclinical models of various cancers however their biological role in MB remains unknown. The aim of this study is to disclose the identity and quantity of MB-related extracellular microRNAs in culture medium (CM) of three MB cell lines as well as in the cerebrospinal fluid (CSF) of human MB patients. Among the large number (1254) of extracellular microRNAs detected in MB-CSF, 441 were over-expressed. 86 out of these 441 microRNAs were differentially expressed in CSF of MB patients compared to controls (CSF from non brain tumor patients). Amongst the extracellular microRNAs detected in CM of each cell line, 1083 microRNAs were common between the three cell lines tested. Interestingly three out of four microRNAs that are over-represented in CM of metastasis-related MB cell lines D283 and D341 were found to be also over-represented in CSF of MB patients. In conclusion although more samples are required to fully verify these results, our work provides the first evidence for the presence of a significant amount of microRNAs excreted extracellularly by MB cells and raises the possibility that - in the near future - microRNAs could be probed in CSF of MB patients and serve as novel biological markers.

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SMAC Mimetics Kill Acute Lymphoblastic Leukemia through Parallel Induction of Apoptosis and Necroptosis

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Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, and although current drug treatment is effective in most cases, there are few effective second-line drugs available for drug-resistant disease. SMAC mimetics (SMs) are an emerging class of novel chemotherapeutics, which activate cell death by inhibiting key anti-apoptotic cellular inhibitors of apoptosis proteins (cIAP1, cIAP2, and xIAP). Using an in vitro screening approach, we show that 20-30% of primary patient-derived ALL cells, including some highly drug resistant patient samples, are sensitive to pre-clinical SMs Birinapant and LCL-161. We demonstrate that highly sensitive ALL samples with IC50s in the low nanomolar range also show elevated TNF receptor 2 (TNFR2) expression, and TNFR2-specific blocking partially rescues SM-induced death. Using pharmacologic inhibitors of either apoptosis (zVAD) or necroptosis (necrostatin-1) we show that SM treatment of primary ALL and ALL cell lines results in parallel induction of apoptotic and necroptotic cell death. Using Lenti-CRISPR mediated gene disruption, we show that SM-induced death requires Rip1-kinase, which activates Rip3/MLKL-dependent necroptosis and/or FADD/caspase-8-dependent apoptosis. Blockade of both pathways through gene disruption or inhibitor treatment was required to restore cell viability after SM treatment. Finally, we demonstrate that SM selectively kills ALL cells in an in vivo xenograft model of refractory ALL. These data show that apoptotic and necroptotic cell death pathways are not mutually exclusive phenomena, but can be activated simultaneously in chemotherapy-resistant ALL. Because SMs can induce both cell death pathways in parallel, these agents have a strong potential for anti-leukemic therapy in refractory childhood ALL.

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A non-apoptotic complex of caspase-8, RIPK1 and cFLIP controls DNA damage response in hepatocytes

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Constantly increased levels of hepatocyte apoptosis is a hallmark of liver tissues from patients with chronic liver diseases (CLD), e.g. HBV or HCV infection, autoimmune disorders or chronic alcohol abuse. We analyzed liver tissue of Mcl-1Δhep mice and TAK1Δhep mice and their intercrossings under steady state, after partial hepatectomy and doxorubicin treatment for apoptosis and proliferation, DNA damage and genetic instability and compared the findings to human liver tissues of patients with CLD and hepatocellular carcinoma (HCC) of various etiologies. Remarkably, apoptosis-induced compensatory hyper-proliferation triggers proliferation-associated DNA damage and genetic instability in hepatocytes and correlated with the risk of HCC development. Interestingly, repair of proliferation- as well as doxorubicin-induced DNA strand breaks, depended on a non-catalytic but scaffolding function of caspase-8 and cFLIP and the kinase activity of RIPK1. Activation of specific DNA repair pathways downstream of the caspase-8/RIPK1 complex were found. Our data indicates that liver tumor development is determined by the (accumulated) amount of liver damage in mice and men. The findings suggest that a sequence, initially induced by apoptotic hepatocyte death, triggers consecutive hyper-proliferation, DNA damage response and genetic instability. Furthermore, we discovered a novel non-apoptotic function of caspase-8 as well as cFLIP and RIPK1 (indicative for the ripoptosome complex) in DNA repair of hepatocytes, potentially preventing the malignant transformation of hepaotcyte and tumorigenesis upon DNA damage.

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The Impact of Innate Lymphoid Cells on IL-12-mediated Tumor Suppression

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Malignant melanoma is a particularly aggressive tumor due to its propensity to metastasize and is the cause of most skin cancer-related deaths worldwide. The cytokine interleukin-12 (IL-12) has consistently been demonstrated to possess potent tumor-suppressing activity in preclinical melanoma models. Local IL-12 delivery results in strong anti-tumor response mediated by RORγt-dependent innate lymphoid cells (ILCs)(1). Our goal is to determine the molecular mechanism underlying the IL-12-mediated tumor suppression by ILCs. Recently, we found evidence that RORγt-fate map(fm)+ ILCs from different organs possess distinct tumor-suppressive potential. RORγt-fm+ ILCs resident in the spleen are able to suppress tumor growth in an IL-12-dependent manner, while RORγt-fm+ ILCs derived from the lamina propria of the small intestine do not. In line with this, splenic RORγt-fm+ ILCs are highly responsive towards IL-12 stimulation as we observe elevated IL-12 receptor expression in splenic RORγt-fm+ ILCs, while small intestinal RORγt-fm+ ILCs express higher levels of the IL-23 receptor. We observed a distinct cell surface expression pattern, and higher levels of IFN-γ production upon IL-12 stimulation of splenic compared to small intestinal RORγt-fm+ ILCs which support this hypothesis. To further identify and characterize molecular targets of IL-12 signaling on RORγt-fm+ ILCs, we will delineate the molecular 'signature' of tumor-suppressive ILCs in response to IL-12 by transcriptome analyses. This way, we plan to identify potential target genes involved in the tumor-suppressive function of RORγt-fm+ ILCs. Taken together, the results of this project will shed light on the mechanisms underpinning ILC-driven tumor suppression.

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Thrombopoietin but not G-CSF Induces Proliferation of Dormant Hematopoietic Stem Cells

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Hematopoiesis is sustained by hematopoietic stem and progenitor cells (HSPCs) in bone marrow (BM), both of which populations express cell-surface receptors to adapt to demand of blood cell production upon hematopoietic challenge. Despite the clinical use of hematopoietic growth factors, little is known about their influence on HSC behaviour. To evaluate their effect on HSC turnover in vivo, we employed CFSE-based single cell divisional tracking(1). Non-irradiated mice were transferred with HSC containing CFSE-labelled Lin-c-Kit+Sca-1+ cells (LKS) and injected with a TPO mimetic, Flt3L, G-CSF, and a CXCR4 antagonist. BM analysis 3 weeks later showed that most of phenotypic HSCs (LKSFlt3-c-Mpl+) were found in a 0-divided fraction in PBS treated animals, while TPO mimetic treatment induced >2 divisions in all HSCs. Flt3L and CXCR4-antagonist had no impact on 0-divided HSCs , while G-CSF reduced dormant HSC numbers in BM. Subsequent cell isolation and transplantation into lethally irradiated mice revealed that while almost all HSCs were contained in 2-4x divided cells in TPO mimetic treated mice, PBS- and G-CSF-treated BM showed HSC activity only in slow 0-1x dividing cells, but not at all in >2x divided cells. Similarly, immunodeficient mice(2) transplanted with human HSPCs revealed that phenotypic human HSCs proliferated and expanded upon TPO mimetic treatment as compared to PBS control. These results demonstrate that activation of TPO signalling recruits dormant murine HSCs into proliferation with maintaining self-renewing potential, whereas G-CSF does not affect HSC cycling. Analysis of human HSCs showed similar results in a xenograft model, suggesting a conserved mechanism across species.

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Biological relevance and therapeutic potential of the hypusine modification system

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Hypusine modification of the eukaryotic initiation factors eIF-5A1 and eIF-5A2 is emerging as a crucial regulator in cancer, infections and inflammation. Although its contribution in translational regulation has been sufficiently demonstrated, its biological role in higher eukaryotes remains poorly understood. To establish the hypusine modification system as a novel platform for therapeutic strategies, we aimed to investigate its functional relevance in mammals by generating and using a range of new knockout mouse models for the hypusine modifying enzymes DHS and DOHH as well for eIF-5A2. We uncovered that homozygous depletion of DHS or DOHH causes lethality in adult mice with different penetrance compared to haploinsufficiency. Our findings revealed that altered cellular localization of eIF-5A might explain the variations between Dhs -/- and Dohh -/- phenotypes. Remarkably, our results also demonstrate that the cancer-associated isoform eIF-5A2 is dispensable for normal development and viability. Together, our results provide first genetic evidence that the hypusine modification in eIF-5A is crucial for homeostasis in mammals. Moreover, these findings highlight functional diversity of the hypusine system compared to lower eukaryotes and indicate eIF-5A2 as a valuable and safe target for therapeutic intervention in cancer.

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Relationships between immunosuppression, stemness and drug resistance in ovarian tumors

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Ovarian cancer (OC) remains the most lethal gynecologic malignancy due to its late diagnosis and resistance to chemotherapy. The identification and validation of novel multi-biomarker panels is crucial for early detection and clinical monitoring of the disease. Potential biomarkers could arise from tumor microenvironment, which is known to promote and enhance cancer progression. This study seeks to determine the relationship between drug resistance, stemness and immunosuppressive properties in OC patients. Tumor and blood samples were collected from 35 women with serous epithelial OC and subjected to further analysis. Flow cytometry was used to phenotype immunosuppressive T lymphocyte subpopulations in peripheral blood, to determine tumor leukocyte infiltration and to evaluate the expression of a panel of stemness-associated and drug resistance markers in tumor cells. Immunosuppressive enzymes in serum and tumor lysate were quantified by ELISA method. qPCR was used to quantify the relative expression of drug resistance-associated genes. We observed that expression of all tested markers distributes unevenly in OC patients and may be related to their prognosis. After applying correlation analysis, we found several statistically significant relationships between different elements of immunosuppression, stemness and drug resistance in ovarian tumors. Based on relationship strength we imply that the most relevant markers are IDO, cytotoxic and regulatory T lymphocytes (immunosuppression), ABCC1 and ATP7B (drug resistance), CD44 and CD24 (stemness). Further investigations are necessary to validate the significance of the relationships in larger study populations and determine the prognostic and predictive value of selected multi-biomarker panel.

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Dissecting β-catenin transcriptional outputs in skin development and non-melanoma skin cancer

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The Wnt signalling pathway plays a major role in most developmental processes, as well as in the homeostasis of adult tissues. Aberrant activation of this pathway is associated with cancer formation. One of the key players in this pathway is β-catenin, which serves as a scaffold for a number of transcriptional co-activators and also has a structural role in adherens junction formation. In the skin, complete loss of β-catenin revealed that it was dispensable for development, but required for hair growth. Moreover β-catenin was found to be of crucial importance in the formation of squamous cell carcinoma. We generated a β-catenin mutant mouse strain, which abrogates β-catenin's signalling function but retains its function in adherens junctions. We want to exploit this mutant to clarify the role of β-catenin in skin development and maintenance as well as initiation and progression of non-melanoma skin cancer. We confirm that complete loss of β-catenin leads to severe hair loss, interestingly, blocking only β-catenin signaling transduction results in weaker effects. By combining our genetic model with a genetic model of squamous cell carcinoma, we are able to dissect specifically the signalling role of β-catenin without affecting its function in adherens junctions in skin cancer. Additionally, we are utilizing β-catenin alleles bearing mutations in the N- or C-terminus to investigate which cohort of transcriptional co-activators is important for tumor progression and maintenance. We are also investigating the role of these transcriptional activation branches in skin development. Our experiments will reveal which interaction interfaces are crucial and thus might be suitable for therapeutic intervention.

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Epigenetic shift as a starting point and background for cell transformation: a novel theory of carcinogenesis

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Our recent studies found that non-coding RNAs, hyperexpression (or reactivation) of which is essential for cancer cells, silence: 1) genes encoding DNA repair enzymes as well as other key elements of all DNA repair systems; 2) genes encoding histone deacetylases, histone methyltransferases and de novo DNA methyltransferases [1]; 3) genes encoding nuclear lamina proteins and elements responsible for nuclear lamina-cytoskeleton connections; 4) genes encoding cell adhesion molecules, cytoskeleton components as well as elements of contact inhibition pathways [2]; 5) proapoptotic genes, tumor suppressor genes as well as genes encoding some cell cycle inhibitors; 6) genes counteracting expression of the stem cell reprogramming factors [3]. This leads to genome instability as well as to overall derepression of chromatin. As a result, reactivation of silent mobile genetic elements becomes possible, that causes a positive feedback between DNA damage level and following derepression of mobile genetic elements, leading rapidly to dramatic genome destabilization. Genes of other non-coding RNAs, which counteract the tumor transformation, undergo silencing. Moreover, in view of the genome instability, we speculate, that some of these genes can undergo the target damage (endogenous gene knockout) as a result of mutations in R-loop during transcription or RNA-dependent DNA methylation. This allows epigenetic reactivation (or overexpression) of: 1) genes encoding histone acetyltransferases, histone demethylases as well as components of chromatin remodeling complexes [1]; 2) genes encoding nuclear transporters and components of nuclear pore complex; 3) genes of heteroorganic antigens; 4) genes responsible for cell motility as well as cell anchoring in other tissues [2]; 5) proliferative and antiapoptotic genes; 6) genes encoding stem cell reprogramming factors [3]. Therefore, shifts in non-coding RNA profile can themselves cause cell transformation and facilitate the cancer cell stemness. Following mutations of oncogenes as well as other coding genes, important for transformation, only consolidate their role in carcinogenesis and lead to the tumor progression.

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The methylation profiling of multiple tumor suppressor genes in plasma cell-free DNA of patients with chest radiological findings: NSCLC versus benign tumors – pilot study

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The presence of cell-free DNA (cfDNA) in plasma from non-small cell lung cancer (NSC LC) patients demonstrates promising diagnostic implications as the minimally-invasive liquid biopsy. Recently, we showed promising but clinically not satisfactory sensitivity/specificity of plasma DNA quantification for NSC LC detection. Mean cfDNA level in 66 NSCLC patients (21.5 ng/ml) was significantly higher than in 40 healthy controls (4.5 ng/ml; p=0.000), but did not differ from values observed in 15 patients with non-malignant tumors (23.4 ng/ml; hamartoma, fibrosis, granuloma). Similarly, mean cfDNA integrity index significantly differed NSC LC (4.0) from healthy controls (1.0; p=0.000), but not from non-malignant group (4.0). To improve the diagnostic power of cfDNA analysis, we introduced a simultaneous methylation profiling of 21 tumor suppressor genes (TSGs) in plasma cfDNA from NSCLC versus non-malignant patients using MS-MLPA assay. 25/32 (78%) NSCLC and 8/8 (100%) benign-tumor cfDNA samples presented at least one TSG methylation. APC, ATM, DAPK1, HIC 1, MLH1 and RARβ were the most frequently methylated genes in NSCLC, while MLH1, TIMP3, TP73 – in hamartoma patients. We demonstrated that optimized MS-MLPA assay allows detection multiple methylated TSGs in plasma cfDNA. The MS-MLPA showed good performance in samples with diverse cfDNA concentrations suggesting that methylation detection rate depends on the methylated DNA content in a sample. The TSG methylation in hamartoma patients may indicate an increased risk of lung cancer. The study is on-going.

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pVHL/HIF-regulated CD70 expression is associated with infiltration of CD27+ lymphocytes and increased serum levels of soluble CD27 in clear cell renal cell carcinoma

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CD70, a member of the tumor necrosis factor ligand superfamily, has been shown to be frequently overexpressed in clear cell renal cell carcinoma (ccRCC). The mechanisms of CD70's up-regulation and its role in ccRCC are unknown. In this study, CD70 expression was immunohistochemically analyzed in 667 RCCs and RCC metastases. Von Hippel-Lindau gene (VHL) mutations, expression patterns of VHL protein (pVHL), hypoxia-inducible factor (HIF) α and several HIF targets were studied in tissues and cell lines and correlated with CD70 overexpression. Gene promoter analysis was performed to confirm CD70 as a HIF target gene. Consecutive tissue sections were immunostained to reveal the relation between CD70-expressing RCCs and tumor-infiltrating lymphocytes positive for the CD70 receptor (CD27). CD70-mediated release of soluble CD27 in RCC was assessed by coculture experiments and sera analysis of RCC patients. Findings indicate that elevated CD70 expression occurred in 80% of primary tumors and metastases of ccRCC and correlated with dysregulation of the pVHL/HIF pathway. In vitro analyses demonstrated that up-regulation of CD70 is driven by HIF. Furthermore, CD27+ lymphocytes preferentially infiltrate CD70-expressing ccRCCs. CD70-dependent release of soluble CD27 in cocultures may explain the high CD27 levels observed in sera of patients with CD70-expressing

ccRCC. The combination of lymphocyte infiltration and CD70-expression in RCC was associated with worse patient outcome. Our findings demonstrate that in ccRCC CD70 expression is regulated by HIF as a consequence of pVHL inactivation. Increased serum levels of CD27 suggest the existence of CD70-expressing ccRCC, thus representing a potential serum marker for patients suffering from this disease.

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WIF1 re-expression in glioblastoma inhibits migration through attenuation of non-canonical WNT signalling by downregulating the long non-coding RNA MALAT1

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Glioblastoma is the most aggressive primary brain tumor in adults and due to its invasive nature, cannot be completely removed. The WNT inhibitory factor 1 (WIF1), a secreted inhibitor of WNTs, is systematically downregulated in glioblastoma and acts as strong tumor suppressor. The aim of this study was the dissection of WIF1-associated tumor suppressing effects mediated by canonical and non-canonical WNT-signalling. We found that WIF1, besides inhibiting the canonical WNT pathway, selectively downregulates the WNT/Calcium pathway associated with significant reduction of p38-MAPK phosphorylation. Knock-down of WNT5A, the only WNT ligand overexpressed in glioblastoma, pheno-copied this inhibitory effect. WIF1 expression inhibit ed cell migration in vitro and in an orthotopic brain tumor model, in accordance with the known regulatory function of the WNT/Ca2+ pathway on migration and invasion. In search of a mediator for this function, differential gene expression profiles of WIF1-expressing cells were performed. MALAT1, a long non-coding RNA and key positive regulator of invasion, emerged as the top downregulated gene. Indeed, knock-down of MALAT1 reduced migration in glioblastoma cells, without effect on proliferation. Hence, loss of WIF1 enhances the migratory potential of glioblastoma through WNT5A that activates the WNT/Ca2+ pathway and MALAT1. These data suggest the involvement of canonical and non-canonical WNT-pathways in glioblastoma promoting key features associated with this deadly disease: proliferation on one hand and invasion on the other. Successful targeting will require a dual strategy affecting both canonical and non-canonical WNT pathways.

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Maintenance of human hematopoietic stem cells and hematopoiesis in in vivo engineered bone organs

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Lifelong self-renewal and multilineage repopulation capacity of hematopoietic stem cells (HSCs) is maintained in a specialized bone marrow (BM) microenvironment, the so-called "niche" that provides HSCs with vital factors for their maintenance. However, little is known about the cellular and molecular components of the human HSC niche. We took a developmental tissue engineering approach that allows differentiating human adult BM-derived mesenchymal stem cells (MSCs) into bone organs through ex vivo and in vivo endochondral ossification. Cartilage template ex vivo differentiated from MSCs was implantated into immunodeficient mice, and 4 weeks later, third-party donor cord blood (CB)-derived human CD34+ cells were transplanted to reconstitute human hematopoiesis following sub-lethal irradiation. Histological analysis at 3 months post implantation showed that human MSC-derived ossicles developed vascularization and mature trabecular bone structure. Flowcytometric analysis at 3 months post CB transplantation showed comparable development of human hematopoiesis with phenotypic

HSC in the human ossicles compared to BM. Similar frequency of CD34+ cells with in vitro colony forming capacity were found in the ossicles as in the mouse bone marrow. Our findings indicate that adult BM MSC develop functional bone organs in vivo through an endochondral ossification process that can support engraftment and maintenance of allogenic human HSCs and hematopoiesis. The functional human bone organ engineering that is transplantable and reengineerable will serve as a platform that allows to studying physiology and pathophysiology of human HSC niche in vivo.

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Autologous T helper cells activate B-ALL cells and support their proliferation

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Precursor B acute lymphoblastic leukemia (B-ALL) is the most common childhood malignancy, representing the leading cause of cancer-related death in children and young adults. ALL cells proliferate in the bone marrow, where they receive survival signals from surrounding cells. Beside non-hematopoietic stroma cells, T helper (Th) cells are also present in B-ALL proliferation sites, but the role of these Th cells is currently unknown. In this study, we investigated whether Th cells from ALL patients contribute to ALL cell expansion. Th cells were isolated from patients' bone marrow aspirates and expanded in vitro. Coculture experiments showed that in the presence of autologous Th cells, ALL cells were more activated and upregulated surface molecules such as HLA-DR, adhesion molecules, receptors for T cell help (CD40, CD25) and activation markers (CD38). In addition, ALL cells showed prolonged survival and 3-4 times increased proliferation when cocultured with autologous Th cells. Interestingly, ALL-supporting Th cells had a pro-inflammatory phenotype, producing high amounts of IFN-γ. Preliminary experiments identified IFN-γ and CD40L as key molecules in this malignant interaction. Together, these results suggest that Th cells contribute to the leukemia-supportive microenvironment in ALL by a mechanism that involves soluble as well as membrane-bound factors. Future therapies might involve removal of the leukemia-specific Th cells or blockage of factors that mediate the malignant interaction.

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Sequential pattern recognition receptor stimulation for cancer immunotherapy

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Immunotherapy represents an emerging field in the treatment of cancer, aiming to restore effective antitumor immune responses in patients. To this end, the stimulation of the innate immune system via pattern recognition receptors such as the Toll-like receptor (TLR) family is in clinical use for the top ical treatment of skin tumors (imiquimod; Aldara®). Further TLR agonists are currently developed for clinical use. We established a protocol employing a synthetic TLR7 agonist for systemic treatment that efficiently inhibits the growth of model tumors by taking advantage of the kinetics of receptor sensitivity (Bourquin*, Hotz* et al. Cancer Res 2011). In addition to the precise timing of TLR stimulation, the sequence of triggering different receptors may improve outcome. We therefore investigated the immunological effects of sequential stimulation of different pattern recognition receptor pathways in vitro and in vivo. We found that whereas repetitive triggering of homologous pathways induced immunotolerance. sequential stimulation of heterologous receptor pathways led to enhanced immune responses compared to single stimulations. More precisely, we observed higher cytokine secretion as well as increased activation of cytotoxic T-lymphocytes and differentiation of type-I T helper cells, which are both implicated in anti-tumoral immunity. We further describe the effectors and intracellular signaling events responsible for this improved response Our findings may have direct implications for clinical studies employing innate immune stimulation for cancer immunotherapy.

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A regulatory motif analysis implicates NF-κB1 and HIF-1α transcription factors as molecular mediators of breast cancer metastasis to the brain

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Clinically-relevant metastases in the brain are diagnosed in 25%-30% of patients with disseminated breast cancer. The poor prognosis, and short median survival time (80% mortality within one year), mirror the lack of targeted therapies. Based on the murine 4T1 breast cancer cell line, we recently developed a unique syngeneic and orthotopic model of spontaneous brain metastasis (4T1-BM2). Transcriptome analysis coupled with review of breast cancer patient datasets identified several molecules mediating brain metastasis with potential clinical relevance as therapeutic targets. We performed an Integrated System for Motif Activity Response Analysis to computationally-predict activity of transcription factors. Hypoxia-inducible factor 1-alpha (HIF-1α) and nuclear factor NF-kappa-B p105 (NF-κB1) were demonstrated to be hyper-activated in 4T1-BM2 compared to the non-brain metastatic control cells. Silencing of NF-κB1 in 4T1-BM2 cells reduced their proliferation in vitro and diminished their brain metastatic potential in vivo. The role of HIF-1α in brain metastatic colonization is currently being investigated. We've identified a potentially clinically relevant mediator of brain metastasis in 4T1-BM2 cells of which the expression depends on both HIF-1α and NF-κB1. Silencing of this molecule reduced the 4T1-BM2 brain metastatic potential. To collect further experimental evidence for the involvement of HIF-1α, NF-κB1 and their downstream target in the formation of brain metastasis we are establishing a murine brain metastatic model based on the D2A1 breast cancer cell line (D2A1-BM2). Small molecular inhibitors for our mediators are in clinical development. This raises the possibility of exploring this pathway as potential target for preventing or treating breast cancer brain metastasis in patients.

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Multiparameter ploidy profiling: A powerful tool to investigate the genomics of diploid tumor populations

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Genomic intratumor heterogeneity is an increasingly recognized phenomenon in various solid tumors. However, most methods are not designed to resolve the complexity of mixed cell populations. We have therefore developed the method of multiparameter ploidy profiling (MPP). We applied this technology to tumor specimens from malignant melanoma and from non-small cell lung cancer (NSCLC) patients. MPP involves isolation of nuclei from tissues, multiparameter flow-sorting of tumor populations and profiling genomes using high resolution array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS). As some tumors are diploid by flow, specific lineage markers, e.g. SOX10 (melanoma) and TTF1 (NSCLC) were used to separate these from normal cells. MPP allowed the sorting of different tumor cell populations from within a single tumor specimen, while excluding non-neoplastic contamination in downstream analysis with aCGH and NGS. We separated three different tumor populations from a single primary NSCLC with a TTF1 multiparameter sort. We detected significant differences in copy numbers exemplified by a CDKN2A homozygous deletion only present in the diploid tumor population. Finally, we used population-specific genomic data to infer the clonal relationship and to postulate the evolution of these tumor cell populations within single tumor specimens. We conclude that the power of MPP lies in deciphering the level of intratumor heterogeneity based on pure ploidy sorts. Most importantly, it enables the separation of diploid tumor populations from diploid, benign stromal cells. We also demonstrated that the analysis of diploid tumor populations within

a single biopsy is crucial for the understanding the clonal composition and evolution of a given tumor.

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Targeting metabolic symbiosis to overcome resistance to anti-angiogenic therapy

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Despite the approval of several anti-angiogenic therapies, clinical results remain unsatisfactory. Transient benefits are followed by rapid tumor recurrence associated with increased invasiveness and drug resistance. Understanding the molecular mechanisms underlying drug resistance remains critical to improved therapeutics. Towards this end, Py2T breast cancer cells were injected into the fat-pad of syngeneic FVB/N mice. Following the angiogenic switch, anti-angiogenic treatment was initiated with Nintedanib, a pan-tyrosine kinase inhibitor. Tumor cells from these mice were FACS-sorted and subjected to gene expression profiling. Analysis of the microarray data suggested a metabolic reprogramming of the tumor cells upon 3-weeks treatment, notably through a shift from oxidative phosphorylation to anaerobic glycolysis. Indeed, combinatorial treatment with a glycolysis inhibitor (3-PO) efficiently inhibited tumor growth. Further, while microvessel densities and pericyte coverage were significantly decreased, pimonidazole-staining showed significantly increased hypoxia, indicating that Nintedanib was functional. However, alternation between highly hypoxic, glycolytic areas and normoxic areas in the Nintedanib-treated tumors suggested the establishment of metabolic symbiosis. Such symbiosis was further confirmed by immunostaining for MCT1 and MCT4, monocarboxylate transporters implicated in lactate exchange in glycolytic tumors. Contrary to other studies which demonstrated that resistance to anti-angiogenic therapy is associated with tumor revascularization, our data suggest an alternative mechanism of resistance whereby the tumor cells undergo a metabolic shift to anaerobic glycolysis, allowing them to survive and proliferate even with reduced blood supply. Hence, targeting metabolic symbiosis by inhibiting or downregulating MCT4 might serve as a beneficial strategy to overcome anti-angiogenic therapy-associated resistance.

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Role of MutLγ Endonuclease in Meiotic Homologous Recombination

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Homologous recombination (HR) is a process that repairs double strand DNA breaks to produce either crossover (CO) and/or non-crossover (NCO) products. MutLγ, a heterodimer of the MutL homologues Mlh1 and Mlh3, plays a critical role during meiotic HR(1). Its meiotic function is fully dependent on the integrity of the putative nuclease motif DQHA(X)2E(X)4E, inferring that its anticipated nuclease activity is involved in the processing of joint molecules(2). Here, we expressed and purified yeast MutLγ; show that it is a nuclease that nicks double-stranded DNA. It binds DNA with high affinity showing a marked preference for Holliday junctions (HJ)(3). We also show that it prefers to bind the open unstacked HJ form. This suggests that MutLγ is part of a complex acting on joint molecules to generate crossovers in meiosis. To further elucidate the exact mechanism, we plan to investigate few possible directions: • Genetic evidence shows that proteins such as Msh4-Msh5 (MutSγ) and Mer3 help to stabilize joint molecules and aid in recruitment of MutLγ. Additionally, Sgs1 and Exo1 are also involved in this pathway. We purified these proteins and plan to check their contribution to MutLγ's nuclease activity on HJs. • Cdc5 acts during pachytene exit. Its mutants accumulate joint molecules, show reduced levels of COs and its expression promotes efficient resolution and increase in COs. In accordance, we purified Cdc5 kinase and will look for meiosis specific post-translational modifications of our proteins. • It might be possible that the information to make specifically COs is buried in the double HJ structure itself. We are currently preparing a plasmid-based double HJ substrate that would serve as a more physiological substrate than single HJ.

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Pygopus2 interaction with H3K4me3 is important for malignant progression of breast cancer

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Pygopus2 (Pygo2), an obligatory nuclear component of β-catenin-TCF/LEF transcription regulatory complex functions as a co-activator of Wnt/β-catenin signaling [1]. Recently, Pygo2 has been found to be upregulated in breast cancer and its knockout has been shown to decrease mammary tumor growth. By binding to histone H3 trimethylated lysine (H3K4me3), Pygo2 can also participate in chromatin reading and writing. To investigate the relevance of this interaction in malignant progression of breast cancer, we have generated a knockin mouse model wherein Pygo2 cannot bind to H3K4me3 (PyMT-Pygo2A342E/A342E) [2]. Compared with the Pygo2 WT mice, the PyMT-Pygo2A342E/A342E mice formed significantly smaller mammary tumors and lower incidence of lung metastasis. Immunohistochemical analysis revealed that the mutant mice have significantly lesser number of proliferating cells compared with the control mice. Further, the mutant tumors were also enriched in CK8/18 positive luminal cells and demonstrated elevated Notch signalling. Cell lines derived from primary tumors of the mutant mice had a lower proliferation rate and also did not undergo a significant epithelial to mesenchymal transition in response to TGFβ treatment. Further, while the WT cell lines activated Wnt signaling in response to Wnt3a stimulation, the mutant cells lines were unable to do so. Collectively, our results suggest that Pygo2 interaction with H3K4me3 is critical for malignant breast tumor progression and it might do so by activating the Wnt/β-catenin signalling that is known to support a basal and aggressive fate of mammary cells [3]. Pygo2-H3K4me3 interaction might therefore serve as an attractive therapeutic target for breast cancer.

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Aberrant Ret expression causes mammary tumors and developmental defects during the post-lactational transition

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The receptor tyrosine kinase (RTK) Ret, a key oncoprotein in thyroid carcinomas due to its gain-of-function mutations, has also been implicated in other types of cancers. Recently, Ret copy number gains and mutations have been reported at low frequencies in breast tumors (1). Furthermore, we and others have reported that Ret is overexpressed in about 40% of human tumors and this correlates with poor patient prognosis (2). Using a transgenic mouse model with the MMTV promoter controlling Ret expression in the doxycycline-inducible system, we show that overexpression of wild type Ret in the mammary epithelium produces hyperplasia and mammary tumors displaying a solid morphology that recapitulates features of human

solid ductal carcinoma in situ. Moreover, Ret-induced tumors express ErbB2 and are estrogen receptor positive. Importantly Ret-induced tumors rapidly regress after doxycycline withdr awal indicating that Ret is the driving oncoprotein. Using next generation sequencing we examined levels of transcripts in these tumors. We found that interleukins and ErbB related pathways could contribute to tumorigenesis. It is well known that RTKs, which are implicated in breast cancer, also have roles in normal development. We found that Ret is highly expressed in mid-lactation. Indeed, Ret appears to have a role in the post-lactation transition to involution since when Ret is induced early in lactation we observe enhanced kinetics of involution. The involution is an inflammatory period well known to drive cancer progression. Thus, our results suggest that if Ret expression is deregulated during the lactation-involution transition this might contribute to breast cancer development.

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Biochemical characterization of hMLH1-hMLH3 in meiotic recombination

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In meiotic homologous recombination (HR), exchange of DNA takes place between homologous chromosomes by crossing over. Double Holliday junctions (dHJs) are intermediates of this process and their resolution by nucleases is an essential step for proper chromosome segregation. MLH1-MLH3 (MutLγ) contains a conserved endonuclease motif present in its MLH3 subunit, which is essential for its meiotic function in yeast(1). Thus, MutLγ has been strongly implicated as the primary putative endonuclease responsible for dHJ resolution in meiosis in both yeast and humans. The mechanism of joint molecule (JM) processing by MutLγ represents one of the key questions in the recombination field. Additionally, MSH4-MSH5 is known to be required for stabilization of JM. Recognition and binding by MSH4-MSH5 seems to be pre-requisite for the recruitment of MLH1-MLH3. It was shown that hMSH4-hMSH5 specifically binds to HJs and subsequently as a sliding clamp embraces the arms of the homologous chromosomes(2). Therefore, it is imperative to study the role of hMSH4-hMSH5 in conjunction with hMLH1-hMLH3 in JM processing. Previously, we showed non-specific endonuclease activity of yeast Mlh1-Mlh3 on super-coiled dsDNA3. We also showed specific binding preference of both yeast and human MLH1-MLH3 for HJ and similar substrates(3). Here we demonstrate that hMLH1-hMLH3 also exhibits non-specific endonuclease activity, which shows conserved endonuclease activity of MutLγ in humans. For hMSH4-hMSH5, we have functional protein complex, which binds strongly to HJ and slides along un-blocked arms of DNA substrates as previously reported. In future, we have planned to study the effect of hMSH4-hMSH5 upon hMLH1-hMLH3 endonuclease activity and its DNA binding affinity. Furthermore, we also aim to investigate hMLH1-hMLH3 activity and binding on more specific substrates like dHJ.

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Activin acts on T cells and macrophages to promote Human Papilloma Virus (HPV) 8-induced skin tumorigenesis

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Activins are multifunctional proteins belonging to the TGF-beta superfamily. Results from our laboratory revealed an important role of activin A in the regulation of skin wound repair and carcinogenesis (1). Thus, mice overexpressing this protein in keratinocytes are characterized by accelerated wound healing (2) and increased susceptibility to chemically-induced skin carcinogenesis (3). Our recent findings showed that activin also dramatically accelerates HPV8-induced skin tumorigenesis in mice. This is accompanied by significant changes in various immune cells in the pre-tumorigenic skin. Namely, the number of tumor-protective epidermal gamma delta T cells is reduced by activin, whereas the number of alpha beta T cells, in particular of immunosuppressive Foxp3+ regulatory T cells, is increased. Furthermore, activin overexpression induces macrophage accumulation in the pre-tumorigenic skin. RNA-sequencing showed that the expression profile of these macrophages is strongly affected by overexpression of activin, indicating polarization towards an alternative activation state, often associated with increased carcinogenesis. To assess the possible contribution of macrophages to the development of skin tumors, we are currently depleting these cells to determine if this affects tumorigenesis. Interestingly, we observed elevated activin mRNA levels in human samples of squamous cell carcinoma and in pre-cancerous skin lesions (actinic keratosis). These findings demonstrate that activin that is secreted by pre-malignant and malignant keratinocytes acts on multiple immune cell types and creates a pro-tumorigenic microenvironment. Therefore, inhibition of activin may be a promising strategy for the prevention and/or treatment of epithelial skin cancer.

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Identification of novel therapeutic strategies for targeting hepatocellular carcinoma – A systematic "omics" approach

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Liver cancer is the second leading cause of cancer mortality. Hepatocellular carcinoma (HCC) accounts for 80% of all primary liver cancers. Sorafenib (the only approved drug for advanced HCC) shows limited success in curbing cancer. Thus, there is an increasingly urgent need for novel agents to target HCC. Since almost 45% of HCC have aberrant mTOR signalling, we have generated an mTOR-driven HCC model by liver-specific deletion of the two tumor suppressors PTEN and TSC1 both of which are negative regulators of mTOR signalling. Mice with liver-specific deletion of PTEN and TSC1 (L-DKO) develop HCC within 20 weeks. Histopathological analysis and molecular classification reveal that the L-DKO tumors indeed mimic human HCC. To find novel targetable candidates for HCC, we have undertaken a systematic "omics" analysis of L-DKO-derived tumors. Preliminary analysis of the "omic" data and their integration using a "hotnet-like" approach predicts novel "signalling hubs" previously unexplored as potential therapeutic targets in HCC. Further, in cancer we detect significant upregulation of 346 novel phosphosites corresponding to 150 proteins that lie in major signalling pathways such as VEGF, EGFR, and JNK signalling. Thus, from our study, we hypothesize that targeting these unexplored "signalling hubs" and

novel phosphorylations might aid in identifying novel therapeutic targets for treating HCC.

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hMENA11a contributes to HER3-mediated resistance to PI3K inhibitors in HER2 overexpressing breast cancer cells

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Human Mena (hMENA), an actin regulatory protein of the ENA/VASP family, is overexpressed in high-risk preneoplastic lesions and in primary breast tumors, and correlates with HER2 overexpression and an activated status of AKT and MAPK(1). The concomitant overexpression of hMENA and HER2 identifies breast cancer patients with a worse prognosis(1). Alternatively expressed isoforms, hMENA11a and hMENAΔv6, with opposite functions, have been identified(2). By reverse phase protein assay, we found that the epithelial associated hMENA11a isoform sustains HER3 activation and pro-survival pathways in HER2-overexpressing breast luminal cancer cells. Since HER3 activation plays a key role in cell resistance to PI3K inhibitors(3), we explored whether hMENA11a is involved in these resistance mechanisms. The specific hMENA11a depletion switched off the HER3-related pathway activated by PI3K inhibitors and impaired the nuclear accumulation of HER3 transcription factor FOXO3a induced by these compounds. On the other hand, PI3K inhibitors affected hMENA11a localization and phosphorylation status. At the functional level, hMENA11a sustains cell proliferation and survival in response to PI3K inhibitors whereas hMENA11a silencing increases pro-apoptotic proteins. In 3D cultured breast cancer cells, the depletion of hMENA11a drastically reduced cell viability upon treatment with BEZ235. These results indicate that hMENA11a in HER2-overexpressing breast cancer cells sustains HER3/AKT axis and contributes to HER3-mediated resistance mechanisms to PI3K inhibitors. We propose hMENA11a as a marker of HER3 activation and of resistance to PI3K inhibition therapies, to select patients who can benefit from these treatments. hMENA11a activity may represent a new target for anti-proliferative therapies in breast cancer.

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Sumoylation regulates EXO1-dependent resection at sites of DNA damage

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Processing DNA double-strand breaks by the error-free pathway of homologous recombination (HR) requires the concerted action of a number of factors. Among these, EXO1 and DNA2/BLM are responsible for the ex-

tensive resection of DNA broken ends to produce 3'-overhangs, which are essential intermediates for later HR steps. Here we address the role of post-translational modifications in the control of EXO1 at sites of DNA damage. We show that EXO1 is a SUMO target and that sumoylation affects EXO1 protein stability under basal, as well as DNA damage conditions. We identify an UBC9-PIAS1/PIAS4-dependent mechanism controlling human EXO1 sumoylation in vivo and, using an in vitro reconstituted system, demonstrate conservation of this mechanism in yeast by the Ubc9-Siz1/Siz2. Furthermore, we show physical interaction between the desumoylating enzyme SENP6 and EXO1, promoting EXO1 stability. We provide evidence that sumoylation and ubiquitylation occur sequentially on EXO1 in response to DNA damage and that the former affects recruitment of EXO1 to sites of damage. Finally, we identify the major sites of sumoylation in EXO1 and show that ectopic expression of a sumoylation-deficient form of EXO1 rescues the DNA damage-induced chromosomal aberrations observed upon wt-EXO1 expression. Thus, our study identifies a novel layer of regulation of this DNA damage response protein by elucidating the cross-talk between sumoylation and ubiquitylation, making EXO1 and the pathways regulating its function an ideal target for anti-cancer therapeutic intervention.

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A novel function of CHD4 as epigenetic co-regulator of PAX3-FOXO1 activity in alveolar rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood and, notably, outcomes for patients with highly metastatic alveolar RMS (aRMS) remain dismal. For this reason, alternative treatment modalities are urgently needed to improve overall cure rates and reduce long-term sequelae. Despite a very low mutational burden, and therefore limited number of potential therapeutic targets, the majority of aRMS are characterized by a specific chromosomal translocation generating the chimeric PAX3-FOXO1 transcription factor, as the main genetic alteration. PAX3-FOXO1 is the main driver in aRMS and represents a highly interesting tumor-specific target, as aRMS tumors are addicted to the oncogenic function of the fusion protein. Since transcription factors are considered to be difficult to target, we used a two-step screening approach to identify novel PAX3-FOXO1 interactors crucial for its aberrant transcriptional activity that would allow upstream inhibition of PAX3-FOXO1. We identified the chromodomain-helicase-DNA-binding protein 4 (CHD4) as co-regulator of PAX3-FOXO1 activity and show that CHD4 is mainly required for the activating activity of PAX3-FOXO1. As CHD4 is also expressed in human aRMS tumors, we further investigated the biological role of CHD4 in aRMS. We demonstrate that CHD4 promotes aRMS cell survival in vitro and induces regression of xenograft tumors of aRMS in vivo upon depletion. Since CHD4 has ATPase activity, our data identify CHD4 as a potentially novel drug target in aRMS.

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Kelch-like protein 15 promotes error-free DNA repair by targeting CtIP for proteasomal degradation

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DNA double-strand breaks (DSBs) are repaired by two competing pathways: Non-homologous end-joining (NHEJ) and homologous recombination (HR). Human CtIP promotes DNA-end resection, thereby committing cells to HR, while, at the same time blocking NHEJ. To ensure appropriate execution of DNA-end resection, thereby maintaining genome stability, CtIP is tightly regulated by protein-protein interactions and post-translational modifications. While it has already been established that CtIP phosphorylation by various protein kinases controls its resection activity, the role of CtIP ubiquitination still remains largely elusive. Using a mass spectrometry-based proteomic screen, we have identified human Kelch-like protein 15 (KLHL15), a substrate-specific adaptor for CUL3-based E3 ubiquitin ligases, as a novel CtIP interacting partner. We were able to show that KLHL15 mediates CtIP ubiquitination, leading to its proteasomal-dependent degradation. Furthermore, we discovered that KLHL15 specifically recognizes an evolutionary conserved 'FRY' motif in the C-terminal domain of CtIP. Remarkably, we found that U2OS cells expressing a CtIP-Y842A mutant deficient in KLHL15 interac-

tion display impaired DNA-end resection and defective DSB repair. Moreover, CtIP-Y842A mutant cells exhibit hypersensitivity and massive chromosomal aberrations upon treatment with camptothecin, a chemotherapeutic drug that induces DSBs. Collectively, our work suggests that KLHL15-CUL3 E3 ubiquitin ligase regulates CtIP protein turnover to maintain genome integrity.

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Next generation humanized mice support engraftment of myelofibrosis CD34+ cells

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Engraftment of CD34+ peripheral blood cells from patients with myelofibrosis (MF) in murine xenograft models is poor and is possibly explained by the lack of supportive microenvironmental factors (1). Thrombopoietin (TPO) has been implicated in the pathogenesis of MF (2). Also, the interaction between human hematopoietic cells and SIRPα expressed on mouse macrophages is critical for human engraftment in xenografts (3). We hypothesized that the constitutive expression of human TPO and human SIRPα may promote the development of the human MF clone in mouse xenografts. Purified peripheral blood CD34+ cells were collected from MF patients and intrahepatically transplanted into sublethally-irradiated newborn humanSIRPα-transgenic/humanTPO-knockin Rag2-/- gamma-/- mice (TPO-SIRPα mice). Mice were sacrificed 12-16 weeks after transplantation. Three of six MF samples generated a human bone marrow graft of ≥20%, which was mainly composed of myeloid cells and the presence of blasts in the patient peripheral blood significantly correlated with human MF engraftment. In 2/2 experiments analysed, a JAK2 and a CALR mutation were detected in the bone marrow of engrafted mice transplanted with the respective patient sample. Finally, preliminary evidence suggests that TPO-SIRPα mice are more supportive of human MF engraftment than NSG mice. The xenograft model described here supports robust engraftment of human peripheral blood MF cells and further supports a role for TPO in the pathogenesis of MF. In contrast to previous models, TPO-SIRPα mice strongly promote myeloid rather than lymphoid engraftment. The tight correlation between the presence of peripheral blood blasts and the human MF engraftment potential suggests that human MF stem cells reside in the blast population.

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Mutant HRAS activates RAS and mTOR signaling and sensitizes to MEK and mTOR inhibitors

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HRAS (Harvey-RAS) is a frequently mutated oncogene in cancer. However, mutant HRAS as a drug target has not been investigated so far. Here, we show that mutant HRAS activates the RAS and the mTOR pathway in lung cancer cell lines. HRAS mutations sensitized toward growth inhibition by the MEK inhibitors AZD6244, MEK162 and PD0325901. Further, we found that MEK inhibitors induce apoptosis in mutant HRAS cell lines but not in cell lines lacking RAS mutations. In addition, knockdown of HRAS by siRNA blocked cell growth in mutant HRAS cell lines. Inhibition of the PI3K pathway alone or in combination with MEK inhibitors did not alter signaling nor had an impact on viability. However, combined inhibition of MEK and mTOR reduced cell growth in a synergistic manner. Our results show that HRA S mutations in lung cancer activate the RAS and mTOR pathways which might serve as a therapeutic option for mutated lung cancer patients.

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Two alternatively spliced hMENA isoforms differentially regulate TGF-β1 signalling and epithelial-mesenchymal transition in pancreatic ductal adenocarcinoma

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The splicing program of the actin cytoskeleton regulatory protein, hMENA, is associated with epithelial-mesenchymal transition (EMT), and two alternatively expressed isoforms, hMENA11a and hMENAΔv6, with opposite functions in cell invasion, have been described (1). As yet no data are available on the different role of hMENA isoforms in pancreatic ductal adenocarcinoma (PDAC), one of the most deadly cancers. Using IHC analysis on normal and transformed pancreatic tissues, we show here that hMENA isoforms are not expressed in normal ducts and are detectable at low levels in PanIN lesions. However, 70% of primary tumors expressed high levels of hMENA/hMENAΔv6, while only 23% show high level of hMENA11a isoform, indicating that the lack of hMENA11a is a frequent event in PDAC. The specific silencing of hMENA11a in 'normal' human pancreatic duct epithelial and PDAC cell lines induces down regulation of E-cadherin and β-catenin expression and disrupts cell-cell junctional integrity. Conversely, the overexpression of the mesenchymal-associated hMENAΔv6 up-regulates the mesenchymal marker, Vimentin, and increases the invasive ability of pancreatic cancer cells. TGF-β1, a potent driver of EMT (2) and PDAC progression (3), specifically up-regulates the invasive hMENA and hMENAΔv6 isoforms, but not the anti-invasive hMENA11a. Functionally, hMENA11a counteracts the TGF-β1-induced EMT by regulating TGF-β1 signalling pathways, whereas, hMENAΔv6 favors the TGF-β1-induced EMT. These findings provide new insights on the role of alternatively spliced variants of hMENA in EMT and identify the hMENA splicing program as a promising pathway for the development of new diagnostics and therapeutics in PDAC.

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Drug response profiling to inform individualized treatment approaches in high risk leukemia

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Novel treatment approaches are needed for patients with acute lymphoblastic leukemia (ALL) who respond poorly to current therapies. The genotypic diversity within ALL identified recently by next generation sequencing technologies calls for individualized novel strategies. However, functional correlations of oncogenic lesions with drug response profiles are ill defined

for ALL. Such response profiles may mirror perturbations in relevant cellular programs that could be exploited therapeutically. We have established an imaging-based cell viability analysis platform to generate drug response profiles for primary patient-derived ALL samples co-cultured with mesenchymal stroma cells, expecting to derive functional information. Our procedure integrates high-content screening, newly developed bioinformatics tools and biochemical approaches. We screened a library of 65 compounds for activity in 37 precursor B-ALL and 23 T-ALL samples including refractory cases. A few agents, including genotoxic drugs, showed activity across all samples and we detected selective activity of particular drugs in distinct patient groups. MLL-rearranged and TCF3-HLF-positive ALL samples as well as a subgroup of T-ALL cases were highly sensitive to the BCL-2-specific BH3-mimetic ABT-199 suggesting BCL-2 dependency for these cases. Multiparametric analyses of in vitro responses predicted in vivo activity of ABT-199 in xenografted mice. Moreover, we could identify synergistic activity of ABT-199 with clinical and preclinical compounds, such as topotecan or epigenetic modifiers. Our drug response profiling procedure contributes to the identification of distinct vulnerabilities in leukemia and may facilitate the selection of candidate drugs for further development into clinical application.

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Hif-2a promotes degradation of mammalian peroxisomes by selective autophagy

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Peroxisomes play a central role in lipid metabolism and their function depends on molecular oxygen. Low oxygen tension or von Hippel-Lindau (Vhl) tumor suppressor loss is known to stabilize hypoxia-inducible factors alpha (Hif-1a and -2a) to mediate adaptive responses, but whether peroxisome homeostasis and metabolism are interconnected with Hif-a signalling remains unknown. By studying liver-specific Vhl, Vhl/Hif1a, and Vhl/Hif2a knockout mice, we demonstrate a regulatory function of Hif-2a signalling on peroxisomes. Hif-2a activation augments peroxisome turnover by selective autophagy (pexophagy) and thereby changes lipid composition reminiscent of peroxisomal disorders. The autophagy receptor Nbr1 localizes to peroxisomes and is likewise degraded by Hif-2a-mediated pexophagy. Furthermore, we demonstrate that peroxisome abundance is reduced in VHL-deficient human clear cell renal cell carcinomas with high HIF-2a levels. These results establish Hif-2a as a negative regulator of peroxisome abundance and metabolism and suggest a mechanism by which cells attune peroxisomal function with oxygen availability.

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Establishment of Langerhans cell histiocytosis model carrying B-RAFV600E mutation in humanized mouse model

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Langerhans cell histiocytosis (LCH) was recently reconsidered as a neoplastic disorder due to the high frequency of mutations detected in the MAPK pathway-related genes. Also the somatic mutation in BRAF gene (B-RAFV600E) was detected in more than 50% of the LCH lesions. The high frequency of B-RAFV600E mutation in LCH disease indicates a central role for this kinase in the pathogenesis of this malignancy. However the cellular origin of LCH is still unclear. Recent studies reveal that the expression of B-RAFV600E in distinct hematopoietic compartments defines the stage and the clinical risk in LCH disease; whereas the expression of B-RAFV600E in differentiated

dendritic cells (DC) resembled low risk disease, the expression of this mutant in DC precursors was associated with the high risk disease and involvement of risk organs. The aim of this study is to create xenograft mouse model, in order to identify the blood-borne LCH-initiating cell. We recently generated MISTRG mice that express multiple human myeloid cytokines and might be suitable for the development and support of myeloid neoplasia. Here we show that the expression of B-RAFV600E constitutively activates the MAPK kinase pathway by measuring the phosphorylation status of Erk1/2 proteins. Also our preliminary in vivo data reveal that the lentivirally-infected CD34+ cells harbouring control vector or B-RAFV600E engraft and proliferate successfully in 3rd generation of humanized mice and the rate of engraftment of BRAFV600E cells seemed to be higher than in the mice transplanted with control vector. The establishment of LCH-like disease probably acquires longer time.

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The IL-33/ST2 pathway contributes to intestinal tumorigenesis in humans and mice

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Colorectal cancer (CRC) develops through a multistep process and is modulated by inflammation. However, the inflammatory pathways that support intestinal tumors at different stages remain incompletely understood. Interleukin (IL)-33 signalling plays a role in intestinal inflammation, yet its contribution to the pathogenesis of CRC is unknown (1). Using immunohistochemistry on 713 resected human CRC specimens, we show here that IL-33 and its receptor ST2 are overexpressed in low-grade and early-stage human CRCs, but not in higher-grade and more advanced-stage tumors. In a mouse model of CRC, ST2-deficiency protects from tumor development. Moreover, bone marrow chimera studies indicate that engagement of the IL-33/ST2 pathway on both the radio-resistant and radio-sensitive compartment is essential for CRC development. Mechanistically, activation of IL-33/ST2 signalling compromises the integrity of the intestinal barrier and triggers the production of pro-tumorigenic IL-6 by immune cells. Together, these data reveal an essential role of IL-33/ST2 signalling for early CRC. They also suggest that therapeutic blockade of the IL-33/ST2 axis might halt CRC progression in its earliest stages.

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Role of TGF-β superfamily in melanoma progression

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The progression of melanoma from localized to invasive disease is often linked with the switch from tumor suppressive to oncogenic pattern of TGF-β signaling. As for other cancer types, TGF-β signaling cascade functions as a tumor suppressor pathway in early carcinogenesis, mainly through its broad anti-proliferative potential, whereas at later stages it puzzlingly favors tumor progression either via direct effects on tumor cell aggressiveness or indirectly by modulating tumor microenvironment responses. However, the mechanisms underlying this dual behavior are largely unknown which often precludes the efficient development of therapies. To clarify the function of TGF-β signaling throughout melanoma progression in vivo, we conditionally ablate (cKO) well-known signaling cascade components in order to understand gain/loss of functions of canonical signaling early and late tumorgene sis in melanoma murine model. Mice having Cre-mediated early Smad7 (a negative key regulator of the TGF-β signaling cascade) ablation developed primary tumors with increased nuclear pSmad2 positivity compared to control tumors although no significant difference were seen in primary tumor numbers. Strikingly, Smad7 ablated mice developed massively increased numbers of distant metastases (mainly in lung, liver, and spleen), whereas control mice showed melanoma-free survival after 5 months. These results indicate that activation of the TGF-β pathway is essential for distant metastasis of melanoma cells already at early stages of melanoma formation. Further, patient survival analysis based on TCGA covering more than 350 cases of cutaneous melanoma revealed that low Smad7 expression is associated with poor prognosis. The strong association between Smad7 levels and disease outcome, along with our functional data obtained in a genetic mouse model of melanoma, suggests tha t Smad7 is a significant risk factor in malignant melanoma patients.

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IL-33 signalling contributes to the pathogenesis of myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPNs) are characterized by the clonal expansion of one or more myeloid lineages. In most cases, proliferation of the malignant clone is ascribed to defined genetic alterations (1). MPNs are also associated with aberrant expression and activity of multiple cytokines (2,3). However, the mechanisms by which these cytokines contribute to disease pathogenesis are poorly understood. Here, we reveal a non-redundant role for steady-state IL-33 in supporting dysregulated myelopoiesis in a murine model of MPN. Genetic ablation of the IL-33 signalling pathway was sufficient and necessary to restore normal hematopoiesis and to abrogate MPN-like disease in a mutant strain null for the inositol phosphatase SHIP. Stromal cell-derived IL-33 stimulated the secretion of cytokines and growth factors by myeloid and non-hematopoietic cells of the bone marrow, resulting in myeloproliferation in homozygous SHIP mutants. In addition, JAK2-V617F-dependent MPN was delayed in mice lacking IL-33 in radio-resistant cells. In human bone marrow, we detected increased numbers of IL-33-expressing cells specifically in biopsies from MPN patients at diagnosis. IL-33 promoted cytokine production and colony formation by primary CD34+ hematopoietic stem/progenitor cells. Moreover, IL-33 improved the survival of JAK2-V617F-

positive cell lines. Together, these data indicate a central role of IL-33 signalling in the pathogenesis of MPNs.

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Combined treatment of neuroendocrine tumours with Lu-177-Exendin-4 and mTOR Inhibitor: a long-term preclinical study

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Peptide receptor radionuclide therapy (PRRT) and the mTOR inhibitor Everolimus are both established therapies for the treatment of well-differentiated pancreatic neuroendocrine tumors (pNET). Everolimus is also thought to have a radiosensitizer effect. The purpose of this study was to evaluate the potential benefits and risks of a combined treatment with Everolimus and PRRT in a pre-clinical setting using the transgenic Rip1Tag2 pNET mouce model. Rip1Tag2 mice were treated twice with Lu-177-Exendin-4, Everolimus 5mg/kg daily or every third day and combination of both (n=10 for each group planned). One series was carried out over 20 days, a second series over a maximum of 9 months. Besides biodistribution, tumour size and survival, AKT/mTOR-pathway activity were analysed. Preliminary long-term results showed minor prolonged survival for PRRT alone (37.8+/-11.9 days) over control (27.3+/-5.9 days). Everolimus (73.1+/-14.1 days) as well as the combined treatment (> 50 days) clearly extended survival. While treatment over 20 days with Everolimus daily lead to reduced tumour weight (41.8+/-40mg; control 73.4+/-26mg), Everolimus given every third day did not alter tumour weight (81.5+/-44mg). Combination of Everolimus daily and PRRT showed high synergistic potential (7.8+/-10mg). Everolimus decreased the mTOR pathway, but only when given daily, whereas PRRT slightly actived it. These results demonstrate efficacy of both treatments alone and the potential of combined treatment. Intermittent administration of Everolimus was not effective, which explains previous contrary results published(1).

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Vincristine-loaded Liposomes for Targeted Rhabdomyosarcoma Delivery

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and displays a highly aggressive behavior. Although current treatments, including surgery and chemotherapy, can achieve good response rates, late side effects represent a heavy burden for cancer survivors. A cyclic peptide with strong affinity for RMS in vitro and in vivo has been identified to selectively deliver drugs to the tumor [1]. The aim of the present work is to develop a long-circulating anticancer liposomal formulation, decorated with the RMS-specific peptide. Such vesicles will selectively recognize furin, a proprotein convertase, overexpressed on the surface of RMS. The targeted liposomes were prepared by incorporating 5 mol% of an N-hydroxysuccinimide (NHS) ester of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxyPEG-2000] (DSPE-PEG-NHS) chemically coupled to the selected

peptide. The best coupling conditions were found to take place in DMSO with a 5 times excess of DSPE-PEG-NHS vs. RMS-peptide. Vincristine (VCR), one of the drugs for first line RMS treatment, was used in this study as therapeutic agent and was encapsulated into 130-nm liposomes following a transmembrane pH-gradient procedure. VCR was efficiently encapsulated in both targeted and unlabeled liposomes and a release of 20% of the entrapped drug from the peptide-decorated vesicles within 24 h showed a slow permeation of VCR across the lipid bilayer. The selective binding of the RMS-specific liposomes are to be tested in vitro in RMS cell lines overexpressing furin and in vivo in tumor-bearing mice. This work was financially supported by the Monika Anna Müller Grant, the Phospholipid Research Center, and the Olga Mayenfisch Foundation.

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The Bcl9-β-Catenin interaction is required for Lgr5+ cancer stem cell maintenance in murine colorectal cancer models

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Mutational activation of Wnt signalling is a key oncogenic event in most colorectal cancers (CRC), but approaches targeting the Wnt pathway downstream of driving mutations have proved challenging (1). Bcl9 and Bcl9l (Bcl9/9l) are components of the Wnt/β- catenin transcriptional activation complex that promote metastasis, whereas inhibition of their interaction with β-catenin prevents xenograft progression (2). We showed previously in a mouse chemical carcinogenesis CRC model that ablation of Bcl9/9l results primarily in strong down-regulation of genes associated with intestinal stem cells, without affecting normal intestinal homeostasis (3). Herein, we assessed the potential clinical relevance of targeting the Bcl9/9l-β-catenin interaction, which is of moderate affinity and appears drugable. We found that induced ablation of Bcl9/9l in pre-established murine tumors recapitulates the phenotype of tumors with constitutive Bcl9/9l deletion, which we confirmed in a genetic model of CRC driven by APC and KRAS mutations. In these two independent Wnt-activated CRC models preventing the interaction of Bcl9/9l with β-Catenin in established tumors rapidly resulted in the loss of stemness and mesenchymal traits and concomitant induction of a more differentiated phenotype. This loss-of-Bcl9/9l phenotype depended on the ability of Bcl9/9l to bind β-Catenin. Our findings suggest that the Bcl9/9l-β-Catenin interaction might represent a promising target for pharmacological inhibition.

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Investigating the Mechanism of Action of Ingenol Mebutate in Keratinocytes and Squamous Cell Carcinoma

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Squamous cell carcinoma (SCC) of the skin is the second most common human skin cancer and the second-leading cause of skin cancer death in the US. Recently, a new drug for topical treatment of actinic keratosis, the precursor of SCC was approved. The active compound of the drug is ingenol mebutate (IM) and has the great advantage of being applied for only two to three days and causes only a short period of local skin reactions compared to other treatments. The mechanism of action has been shown to be inducing cell death and eliciting a specific PKC-dependent immune reaction. However,

the direct effects of IM on keratinocytes and AK/SCC cells have not been elucidated. In this project we are investigating pathways that were found to be involved in the mechanism of action of ingenol mebutate.

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3D Cell-based diagnostic tool identifies novel anti-metastatic therapy targets in medulloblastoma

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The inherent tendency of cancer cells to form metastases distant from the primary site necessitates the management of cancer with high dose radiotherapy and non-specific multidrug chemotherapy. This therapy often causes disabling side effects in long-term survivors which highlights the need to develop therapies that target metastasis specifically. We have developed high throughput 2D and 3D cell motility models coupled with high-content phenotype-based microscopy analysis and automated computer-assisted quantification to study the interconnections chemical tumour microenvironment parameters (growth factors and cytokines) and cell motility. Using this approach, we are currently interrogating the cell dissemination of the pediatric brain tumour, medulloblastoma (MB). We studied the effects of the most predominant growth factors and cytokines present in the tumour microenvironment of MB and found that hepatocyte growth factor, basic fibroblast growth factor and epithelial growth factor had the maximum influence on MB cell motilty both in 2D and 3D environment in established human MB cell lines and in patient-derived xenografts. Thus, this method aids as a diagnostic tool which provides a finer outlook of growth factor-driven MB cell motilty in patient-derived tumour samples. Conjointly, screening matrices will be adapted to reveal relevant combinations of growth factor pathways promoting MB dissemination in 2D and 3D environment. Further analysis via an inhibitor screen using a selected repertoire of compounds targeting the kinases in signalling pathways pertinent to the growth factors influencing MB cell motlity will facilitate identification of novel targets to selectively treat metastasis.

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Cancer Stem Cells and Tumor Heterogeneity in Renal Cancer

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Clear cell renal cell carcinoma (ccRCC), the most common type of renal cancer, is a very heterogeneous tumor with multiple key driver mutations. However, which tumor subpopulation, driver mutation or stem cell-like ccRCC subpopulation regulates tumor spreading and initiation of metastasis remains unresolved. To elucidate these open questions, primary ccRCC cell lines were established for studying the mutational profile, the expression pattern of RNA and protein level in relation to in vitro and in vivo characteristics. Seventeen RCC cell lines were first studied in terms of their mutational genetic profile by next generation sequencing. Furthermore, the protein expression of PBRM1 and BAP1 was assessed by immunohistochemistry and western blot and was found to be correlated with the mutational status of the corresponding genes. To establish the sphere assay, four RCC cell lines were tested in relation to different conditions: seeding cell number, medium recipe and low attachment plates. Only the A-498 cell line showed sphere formation capability. So far, eight RCC primary cell lines have been established and maintained in culture. Four of them have already been analyzed by Sanger sequencing to obtain information about the genetic identity of each primary cell line to the primary tumor from which they were derived. In addition, immunohistochemical markers such as PancK a and b, BAP1, PBRM1 and the cancer stem cell marker CD105, revealed similar expression patterns in both primary cell lines and primary tumor tissue. Finally, different morphological and cell growth characteristics were evaluated while culturing. A decreased proliferative rate was observed by increasing passage numbers. In conclusion, cell lines represent a valuable tool to characterize

and investigate the genotype and phenotype of RCC in vitro. This may also help to identify cancer stem cell or cancer cell subpopulations giving rise to chemoresistance and metastatic potential, respectively.

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Effects of hedgehog antagonist in an orthotopic malignant pleural mesothelioma rat model

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The hedgehog (Hh) signalling pathway has been found to be activated in malignant pleural mesothelioma (MPM), leading to poor prognosis. We investigated effects of vismodegib, a FDA-approved Hh antagonist, in MPM employing an orthotopic immunocompetent rat model. Sarcomatoid rat MPM cells transfected with luciferase (IL45-luc) were implanted subpleurally in Fischer rats. When tumors appeared (bioluminescence(Bli) monitoring), vismodegib was administered daily (100 mg/kg, peroral; n=6) for 6 days while control group (n=6) received vehicle alone. After the treatment began, Bli was monitored every second day. At day 6, tumor volume was measured by magnetic resonance imaging (MRI) and tumors were harvested and evaluated for Hh target genes expression by real time PCR and immunohistochemistry. Tumors derived from IL45-luc express Hh signaling ligand (Desert hedgehog) and receptors (Ptch1, Smo). In the treated group, tumor volume measured by MRI was significantly reduced compared to control (p=0.03), whereas only a non-significant trend of tumor growth inhibition could be observed by Bli (p=0.15). Nevertheless, Ki-67 staining confirmed reduced proliferation of tumors in the treated group (p=0.006). The treatment with vismodegib slightly suppressed protein expression of the well characterized Hh target gene GLI1 in the tumors. Interestingly, more pronounced GLI1 down regulation was observed in the stromal compartment. Furthermore, we observed significantly reduced expression of fibronectin and vascular endothelial growth factor (Vegf) in the treated group. We demonstrated activated Hh signaling in the rat MPM model. Treatment with vismodegib reduced tumor volume potentially by affecting stroma as reflected by down-regulation of fibronectin and Vegf.

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The Dual-Specificity Phosphatase DUSP4 is a Novel Tumor Suppressor and Important Negative Regulator of JNK Signaling in Diffuse Large B Cell Lymphoma

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The aim of this study was to investigate the functional relevance of CpG island hypermethylation in diffuse large B-cell lymphoma (DLBCL) pathogenesis. We performed whole-genome methylation analysis of DLBCL and healthy control samples and integrated these datasets with RNA expression data. This analysis identified candidate genes, which are expressed in healthy B cells, epigenetically silenced in DLBCL samples and re-expressed after global DNA de-methylation. In-depth functional analysis of 30 candidate genes revealed the dual-specificity phosphatase 4 (DUSP4) as an important tumor suppressor in DLBCL. DUSP4 is a negative regulator of MAP kinases and its expression is lost in the majority of DLBCL cases either through promoter hypermethylation or deletion of its genomic locus. Ectopic expression of wild-type DUSP4, but not of a phosphatase-dead mutant, in DLBCL cell lines strongly reduced cell viability. This is correlated with dephosphorylation of JNK1/2, but not any of its other known kinase substrates, suggesting that JNK is the preferred target of DUSP4 in lymphoma cells. Pharmacological inhibition of JNK mimics the phenotype of DUSP4 re-expression and strongly reduces lymphoma cell viability in vitro and in vivo. Strikingly, low DUSP4 ex-

pression correlates significantly with inferior survival in three DLBCL patient cohorts analyzed. These combined results suggest that active JNK signalling is important for DLBCL cell survival and that epigenetic dysregulation of the JNK pathway, through loss of DUSP4 expression, contributes to DLBCL pathogenesis. Concluding, we propose JNK as a promising new target for pharmacological intervention in DLBCL, particularly in synergistic lethal combination with Bruton's tyrosine kinase inhibitors.

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T-cadherin as a novel determinant of prostate cancer progression: expression in human tissue and modulation of growth factor receptors and beta-catenin pathways

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T-cadherin is an atypical glycosylphosphatidylinisotol-anchored member of the cadherin superfamily of adhesion molecules. We have previously demonstrated that T-cadherin overexpression in malignant (DU145) and benign (BPH-1) prostatic epithelial cells caused acquisition of a migratory phenotype and promoted spheroid invasion in 3-dimensional matrix, while silencing in BPH-1-rescued epithelial morphology and decreased invasion. These effects were mediated by growth factor receptors EGFR and IGF-1R. The aim of this study was to evaluate T-cadherin expression in human prostate cancer samples, and identify intracellular signalling targets of T-cadherin in prostate cancer cells. Quantitative PCR (qPCR) examination of 101 tissue samples demonstrated that T-cadherin mRNA levels did not differ between benign prostate hyperplasia and prostate adenocarcinoma specimens. However, in adenocarcinoma specimens T-cadherin expression correlated significantly and positively with the Gleason score. Tissue microarray analysis showed that T-cadherin protein was absent or weakly expressed in benign tissue, while some prostate adenocarcinoma samples displayed very strong T-cadherin expression. Lentivirus-driven T-cadherin overexpression did not induce epithelial-to-mesenchymal transition (EMT) in DU145 cells evaluated by expression of EMT markers using qPCR and Western blotting. Confocal microscopy followed by quantitative analysis of reconstructed 3-dimensional images of DU145 monolayers and acini grown in Matrigel showed that T-cadherin overexpression caused up-regulation of total and active beta-catenin levels. Cell fractionation confirmed higher beta-catenin levels in total lysates, membrane and nuclear fractions of T-cadherin-overexpressing DU145 cells. Our data suggest that T-cadherin upregulation may promote prostate cancer progression and dissemination via modulation of growth factor receptor and beta-catenin pathways.

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Gastrokine as a novel potential biomarker for premalignant pancreatic lesions

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Gastrokine (GKN) is a protein synthesized by cells of the gastric mucosa and shows growth factor or 'cytokine-like' activity toward gastric epithelial cells. GKN expression is believed to be almost exclusive to the stomach, except for trace levels in the uterus, placenta and duodenum. In the stomach, GKN expression is confined to the gastric epithelium, where individual paralogs (GKN1-3) manifest remarkable cell-type-specific localization on different mucus-secreting epithelial lineages. GKNs can regulate gastric epithelial homeostasis and possess tumor suppressive activity. A whole genome microarray analysis of a mouse model for pancreatic carcinogenesis (KrasxPtf1a) revealed striking GKN upregulation in the pancreas. We aimed to investigate GKN expression and function during pancreatic carcinogenesis. Microarray results were confirmed by RT-PCR. GKN expression was visualized by immunohistochemistry. The presence of GKN in mouse pancreatic juice was determined by proteomic analysis. Gene expression analysis showed high GKN expression during mouse pancreatic carcinogenesis and in human patients with pancreatic malignancies. GKN expression in normal pancreas was undetectable. Immunohistochemistry revealed strong GKN expression in premalignant PanIN lesions. GKN1 was abundant in the cytoplasm of dysplastic

epithelium, whereas GKN2 was localized on associated inflammatory cells. Elisa and proteomic analysis in mouse confirmed the secretion of GKN1 into pancreatic juice, but not serum. In conclusion, we identified for the first time GKN expression in neoplastic human and mouse pancreatic tissues. GKN expression is specific for premalignant PanIN lesions and it is secreted into the pancreatic juice during pancreatic carcinogenesis. Therefore, GKN could serve as a potential biomarker for early pancreatic pre-neoplastic lesions.

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The ubiquitin-specific peptidase 15 (USP15) suppresses the proliferation of human glioblastoma cell lines via stabilization of HECTD1 E3 ligase

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Glioblastoma (GBM) is the most aggressive malignant brain tumor with median survival of 15 months. Previous gene expression studies from human GBM samples in our laboratory revealed ubiquitin-specific peptidase 15 (USP15) significantly associated with genomic deletion in 25% of the cases. USP15 removes ubiquitin moieties and stabilizes proteins. Previously, USP15 has been suggested to have a tumor suppressor function via its substrates APC and Caspase 3. Therefore, we established GBM cell lines that stably express USP15 WT or its catalytic mutant. USP15 expression reduces colony formation and growth in soft agar assays by inhibiting cell cycle progression. In order to identify the molecular pathways in which USP15 is implicated, we aimed at identifying protein-binding partners using the GBM cell line LN-229 by mass spectrometry. Eight new proteins interacting with USP15 were identified, and one of them is the HECTD1 E3 ligase. The murine homologue of Hectd1 promotes the APC-Axin interaction to negatively regulate the Wnt pathway. We could show that USP15 can interact and de-ubiquitinate HECTD1 in the LN-229 cell line. Depletion of USP15 led to a decrease of HECTD1 in GBM cell lines. Moreover stable expression of HECTD1 in LN-229 inhibits colony formation in soft agar assays and dampens cell cycle progression too. Taken together, the data support the notion that USP15 may have a tumor suppressing function via the stabilization of HECTD1. Further investigations aim at uncovering the pathways that are affected by these two proteins and their potential as therapeutic targets for a subpopulation of GBM patients.

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MiR-Score: A 6-microRNA signature for prognosis in malignant pleural mesothelioma

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Prognosis for malignant pleural mesothelioma (MPM) is poor, and predicting the outcome of treatment is particularly difficult. We report here identification of a 6-microRNA signature, the miR-Score, as a novel prognostic factor for mesothelioma patients (1). Candidate microRNAs from microarray profiling of tumour samples categorized as long (median: 53.7 months) and short

(median: 6.4 months) term survivors were validated in 48 tumour samples from patients undergoing extrapleural pneumonectomy (EPP). Kaplan-Meier log ranking was used to further explore the association between microRNA expression and overall survival (OS). A microRNA expression signature for prediction of ≥20 months OS was built using binary logistic regression modelling. Performance of the signature (miR-Score) was evaluated by receiver operating characteristics curve analysis. The miR-Score was then independently validated in 43 tumour samples from patients undergoing palliative pleurectomy/decortication (P/D) surgery The miR-Score including 6 microRNAs, miR-21-5p, -23a-3p, -30e-5p, -221-3p, -222-3p and -31-5p, was able to predict a long survival with an accuracy of 92.3% in patients undergoing EPP and an accuracy of 71.9% in patients receiving palliative P/D. Score-positive patients showed increased median overall survival of 23 and 9 months for EPP and P/D, respectively. Hazard ratios for score-negative patients were 4.12 (95% CI: 2.03–8.37, p=0.00001) for EPP and 1.93 (95% CI: 1.01–3.69, p=0.047) for P/D. Furthermore, adding the miR-Score to a set of clinical factors (histology, age, gender) resulted in improved predictive accuracy in the independent validation cohort. This study has identified a novel microRNA signature, the miR-Score, which can accurately predict prognosis of MPM patients.

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RIPK3 affects tumor progression and invasion independent of the necroptotic function

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Receptor-interacting serine/threonine protein kinase 3 (RIPK3) is well known for its ability to regulate programmed necrosis or necroptosis. It is believed to be a defense mechanism against viral infection. Apart from its role in cell death signalling, we have shown that RIPK3 is a potential regulator of multiple cytokines and chemokines. Using the B16 melanoma model, the loss of RIPK3 affected lung tumor burden in a RIPK3 dose-dependent manner. Analysis of immune cell infiltrates into the lung suggests that the immune response to tumor challenge in Ripk3-/- mice was altered and possibly delayed. The cytokine/chemokine profile of lung homogenates in Ripk3-/- mice showed a number of cytokines/chemokines were increased upon tumor nodule formation, such as CCL2, CXCL1 and CXCL2 while others, such as matrix metalloproteinase 9, were significantly down-regulated. Surprisingly, using bone marrow chimeras, we found that the stromal compartment and not the hematopoietic compartment contributed to the reduced tumor burden in the Ripk3-/- mice compared to wild-type mice. Moreover, the kinase activity of RIPK3 was not required for the tumor cell invasion but was for the growth of the B16 tumor nodule. In addition, blood vessel permeability was reduced by the complete loss of RIPK3. Taken together, our data suggest the necroptotic function of RIPK3 is not required for tumor cell invasion, but is important for tumor nodule growth. Moreover, the tissue remodeling of the extracellular matrix for tumor growth and invasion requires a non-necroptotic function of RIPK3. These results highlight a role for RIPK3 beyond cell death and in inflammatory pathways directly influencing disease progression, suggesting RIPK3 inhibitors in development may be tested for reduction of both tumor progression and invasion.

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Towards identification of synthetic lethal interactors of URI1 in 19q12 amplified ovarian cancer cells by a pooled shRNA screen

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Amplification of the chromosomal locus 19q12 has been observed in multiple tumor types including breast, endometrial, gastric, small cell lung and ovarian cancer. The most frequent malignant tumors with 19q12 amplification are high-grade serous ovarian carcinomas which are highly resistant to platinum-based chemotherapy. Multiple genes have been shown to be amplified at 19q12 including URI1, which encodes an unconventional member of the prefoldin (PFD) family of molecular chaperones (1). Existing evidence suggest that URI1 acts as a regulator of the apoptotic response downstream of the mTOR-S6K1 signaling pathway (2). Moreover, its excessive production in URI1-amplifed ovarian canc er cells fuels evasion from apoptosis (3). Based on these results and the fact that within 19q12, URI1 is the only gene

essential for the survival selectively of 19q12 amplified ovarian cancer cells, it has been proposed that URI1 has properties of an addicting oncogene (3). By screening the human kinome for synthetic lethal interactors of URI1, we aim to discover protein kinases that are specifically required for the survival of 19q12-amplified and thus URI1 oncoprotein-addicted ovarian cancer cells. We expect that this approach will provide new therapeutic targets for 19q12-amplified malignant tumors.

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Sensitization to chemotherapy by interfering with MNK pathway in human gliomas

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Current standard-of-care treatment for malignant gliomas includes surgical resection, radiotherapy and adjuvant chemotherapy with temozolomide (TMZ). The cellular response to DNA damage is mediated through multiple pathways that regulate DNA repair, cell cycle arrest, and apoptosis. Previous study indicates that DNA damage response is coordinated by selective mRNA translation mediated by high levels of translation initiation factor eIF4G1 in cancer cells. Kinases closely associated with translation initiation complexes influence their activity and thus, can regulate selective modes of translation. MAP kinase-interacting kinases (MNK) bind to translation initiation factor eIF4G, and phosphorylate the cap-binding protein, translation initiation factor eIF4E. Phosphorylation of eIF4E at Ser209 is required for eIF 4E ability to oppose apoptosis and promote tumorigenesis in vivo. Recently, we analyzed phosphorylation of MNK-specific substrate eIF4E during chemotherapy. We observed increased phosphorylation level of eIF4E indicating high MNK activity in TMZ-treated glioblastoma (GBM) cells. Importantly, depletion of MNK activity using two MNK inhibitors or MNK1-specific knock-down sensitized glioma cells to TMZ. Furthermore, TMZ treatment combined with MNK inactivation inhibited growth of GBM-derived spheres and reduced tumor growth in an orthotopic GBM mouse model. In order to understand how MNK pathways support survival during chemotherapy with TMZ, our most recent study has analyzed putative MNK substrates by quantitative phosphoproteomics. Taken together, our study data indicate an activation of MNK-mediated survival mechanisms via translation regulation in response to glioma chemotherapy.

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Mechanistic Insight Into Replication Fork Reversal Upon Genotoxic Stress

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Topoisomerase I inhibitors such as campthotecin (CPT) have been shown to trap the enzyme on nicked intermediate (TopI cleavage complex –TopIcc) and lead to double strand breaks (DSBs) during replication. Low CPT doses induce replication fork slowing and reversal, which prevents CPT-induced DSBs. Recent work showed that accumulation of reversed forks upon CPT and other genotoxic treatments is modulated by poly(ADP-ribose) polymerase activity, through transient inhibition of the fork restart activity of RecQ1 helicase (1, 2, 3). This project aims to identify cellular factors driving replication fork reversal, combining predictive cell biology assays and single-molecule analysis in vivo. Based on literature study, candidates belong to two main groups, i.e. annealing helicases and factors involved in post-replicative repair were selected. Two of the annealing helicases (SMARCAL1 and ZRANB3) and Ubc13 showed encouraging results in our ongoing investigations and are therefore prime candidates for driving fork reversal upon low doses of genotoxic stress.

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TLR stimulation in tumor-bearing mice leads to an increase of the necrotic area into the tumor

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Toll-like-receptors (TLR) and RIG-like receptors (RLR) are part of the innate immune system, the first defense line against pathogens. This innate immune response can be harnessed for cancer immunotherapy in order to activate anti-cancer immunity or to diminish the tumor-associated immune regulation. Topical treatment based on stimulation of TLR7/8 (imiquimod) is already used in the clinic for the therapy of basal cell carcinoma. In the present study, TLR7 stimulation, TLR3/MDA-5 stimulation and a combination of both were achieved pharmacologically in mice bearing subcutaneous CT26 tumors. After several injections of TLR/RLR ligands, the effects of different treatments were analyzed by histology with a focus on the intratumoral necrotic area. Notably, the TLR3/MDA-5 ligand poly(I:C) and the combination of poly(I:C) and the TLR7 ligand R848 showed an increase in intratumoral necrosis. Mice treated with poly(I:C) had an average of 70% necrosis, mice treated with the poly(I:C)/R848 combination showed 60% necrosis, whereas the necrosis in PBS-treated control mice was only 30% of the total tumor area. Necrosis of the tumor could be could part of the beneficial effect of TLR/RLR targeted antitumor therapy. Investigations on the mechanisms leading to the increase of necrosis in the tumor are ongoing. Indeed, during treatment, hemorrhagic areas have been observed around and in the tumor suggesting an action on the vasculature of the tumor. Additional experiments should clarify whether the treatment directly targets endothelial cells or if the mechanism is mediated by activation of innate immune cells present in the microenvironment of the tumor.

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MicroRNA-138 promotes acquired alkylator resistance in glioblastoma

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Glioblastoma is the most aggressive brain tumor in adults with median survival below 12 months. The main reason for tumor recurrence and progression is constitutive or acquired resistance to the standard of care, temozolomide (TMZ)-based chemoradiotherapy (TMZ/RT→TMZ) (1). Here we investigate the role of selected microRNAs (miR) molecules as mediators of alkylator resistance in glioblastoma cells. To this purpose, we performed a microRNA array of three human glioma cell lines with acquired TMZ resistance (2) and assessed several deregulated miR molecules potentially involved in TMZ resistance. We identified miR-138 and miR-192 as significantly up-regulated in two cell lines with induced resistance. Both miR were significantly up-regulated in glioblastoma at failure of TMZ/RT→TMZ in vivo, too. While miR-192 overexpression alone failed to induce TMZ resistance in glioma cells in vitro, overexpression of miR-138 by mimic transfection increased TMZ resistance in glioblastoma cells with low miR-138 expression. Activated leukocyte cell adhesion molecule (ALCAM) emerged as a predicted miR-138 target which was found to be down-regulated as indicated by protein levels in LN-18 and LNT-229 resistant cells. However, silencing of this gene was not sufficient to mimic an acquired TMZ resistance-phenotype achieved by miR-138 over-expression. Altogether, these data define miR-138 as a potential oncogenic miRNA in glioblastoma associated with resistance to therapy and progression. The exact mechanism by which this miRNA acts is still to be elucidated.

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The potential role of HTATIP2 as a Tumor suppressor in Glioblastoma

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In a genome-wide methylation analysis of glioblastoma samples, the HTATIP2 (HIV1 Tat Interactive protein2) gene promotor was found to be differentially methylated. The strong negative correlation between promotor methylation and gene expression makes it an interesting gene to investigate as a tumor suppressor in glioblastoma (GBM). HTATIP2 is an oxido-reductase protein that, in an NADPH dependent manner, inhibits the nuclear importation of proteins through β-Importins. Latest findings in lung and hepatocellular carcinoma suggest that HTATIP2 has roles in delaying EGFR-protein degradation in the early endosomes and hence facilitating a prolonged downstream signalling of EGFR. In this study, we aim to characterize the potential role of HTATIP2 as tumor suppressor in glioblastoma. We have developed GBM cell lines that ectopically over-express HTATIP2 and observed variable outcomes in the growth and anchorage dependent colony formation potential, thus implicating the direct involvement of HTATIP2 in conferring classic tumor suppressor phenotypes in these cells. In a second strategy, we are investigating the effect of HTATIP2 on cellular localization of cancer-relevant nuclear proteins such as EGFR and the DNA repair enzyme MPG. GBM cell lines that endogenously express MPG and EGFR are currently being tested for their cellular localization, in the presence or absence of HTATIP2. We hypothesize that HTATIP2 has a tumor suppressing function, and could potentially block the nuclear translocation of cancer-relevant proteins such as EGFR and MPG, thus hampering their specific functions in the nucleus.

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Cytosolic pH acts as a cellular signal to promote cell growth: Evidence from yeast and mammals

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Nutrients are a major cell growth determinant and regulate conserved signalling pathways to adjust cellular physiology to environmental conditions. Although metabolic function is widely recognized as impacting on health and disease, little is known about the molecular mechanisms of nutrient sensing. In yeast, glucose activates the Ras/PKA pathway and TORC1, which regulate growth through enhancing translation and ribosome biogenesis and suppressing autophagy. Here, we show that cytosolic pH is sensitive to the quality and quantity of the available carbon source (C-source) and correlates with growth under these conditions. Interestingly, genetic analysis revealed that high cytosolic pH is both sufficient and required to activate Ras and TORC1. Moreover, both pathways are activated through the vacuolar ATPase (V-ATPase), which serves as a sensor for cytosolic pH in vivo, through activation of distinct GTPases, Arf1 and Gtr1. Thus, our study provides a molecular mechanism through which cytosolic pH links C-source availability to the activity of signalling networks promoting cell growth. Strikingly, increased cytosolic pH is considered a hallmark of cancer cells, and thus may also be linked to increased cell growth in this system. Indeed, we found that cytosolic pH is regulated in a glucose- and growth factor-dependent manner in retinal pigment epithelial cells and that an increase in cytosolic pH is nec-

essary for proliferation upon growth factor stimulation. Taken together, these data suggest that cytosolic pH might have evolved as a conserved, specific signal regulating nutrient-sensitive signalling pathways to promote growth, with potentially wide spread implications for tumor cell biology.

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Neuropilin-2 modulates metastases promoting CXCR-4/CXCL-12 signalling in colorectal cancer

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It has been shown that neuropilin-2 (NRP-2) is highly expressed in colorectal cancer. Recently, we discovered a previously unkown role of NRP-2 in promoting autophagy, therapy resistance and endosomal maturation in different cancer entities [1, 2]. Endosomal maturation is important for the function of several metastases-promoting receptors such as the chemokine receptor CXC receptor 4 (CXCR-4). This study evaluates the role of NRP-2 in CXCR-4 signalling in colorectal cancer. The expression of NRP-2 and CXCR-4 was detected by flow cytometry in the human colon cancer cell lines SW480, SW620 and Colo320DM. NRP-2 was depleted by RNAi. CXCR-4 signalling cascades were analyzed by immunoblotting for phosphorylated ERK proteins – a downstream target of the CXCL-12/CXCR-4 axis. Chemotactic migration was studied by Boyden chamber assays. NRP-2 expression in lymph node metastases was tested by immunohistochemistry. NRP-2 was expressed in all investigated cell lines and co-expressed with CXCR-4 in the non-metastatic cell lines. NRP-2 silencing by RNAi abrogated the chemotactic migration towards the CXCR-4 ligand CXCL-12. Accordingly, the depletion of NRP-2 modulates the short term activation of ERK-1/2 by CXCL-12. In line with our results, lymph node metastases of colorectal cancer patients showed strong staining for NRP-2. In conclusion, NRP-2 is an important modulator of the prometastatic CXCR-4/CXCL-12 signalling axis in colorectal cancer.

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HOXA13 expression modulates response to Sorafenib in hepatocellular carcinoma

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Despite significant advances in hepatocellular carcinoma (HCC) diagnosis and management, for advanced stages of disease no therapeutic options exist beside the multikinase inhibitor Sorafenib. Recently, we showed that HOXA13 expression in HCC correlates with poor patient survival, metastasis and increased HCC cell proliferation in vitro(1). Here we seek to confirm our data on a larger cohort samples, and to investigate whether HOXA13 modulates Sorafenib response both in vitro and in vivo. A tissue microarray comprising 305 specimens [82 from normal liver, 108 from cirrhotic liver and 115 HCC specimens] has been stained for HOXA13, CK-7, CK-19. Additionally, HOXA13 levels were investigated in specimens from a cohort of 40 Sorafenib-exposed HCC patients. Protein levels were correlated with patients' clinical data, including survival. In vitro experiments to modulate HOXA13 expression (gain and loss of function) were performed using the HCC-derived cell lines: Hep-G2, SNU449 and PLC5. Subsequently, cells were treated with Sorafenib. These studies indicated that HOXA13 is altered in 41% of tested HCCs, confirming our previous results. HOXA13 is associated with poorer outcome and higher tumour grade. Increased HOXA13 expression is linked with stem-progenitor markers, CK-7 and CK19. Furthermore, preliminary data suggest that high HOXA13 expression results in poorer response to Sorafenib treatment. As well, in vitro experiments demonstrate that HOXA13 overexpression results in higher resistance to Sorafenib exposure. Conversely, HOXA13 downregulation sensitize HCC cells to Sorafenib. We conclude that HOXA13 levels predict HCC outcome correlating with a number of tumour features. In addition, our preliminary in vivo and in vitro data suggest that HOXA13 could modulate Sorafenib treatment response.

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Treatment with 5-aza-2\'-deoxycytidine induces expression of NY-ESO-1 and facilitates cytotoxic T lymphocyte-mediated tumor cell killing

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NY-ESO-1 belongs to the cancer/testis antigen (CTA) family and represents an attractive target for cancer immunotherapy. Its expression is induced in a variety of solid tumors via DNA demethylation of the promoter of CpG islands. However, NY-ESO-1 expression is usually very low or absent in some tumors such as breast cancer or multiple myeloma. Therefore, we established an optimized in vitro treatment protocol for up-regulation of NY-ESO-1 expression by tumor cells using the hypomethylating agent 5-aza-2\'-deoxycytidine (DAC). We demonstrated de novo induction of NY-ESO-1 in MCF7 breast cancer cells and significantly increased expression in U266 multiple myeloma cells. This effect was time- and dose-dependent with the highest expression of NY-ESO-1 mRNA achieved by the incubation of 10 µM DAC for 72 hours. NY-ESO-1 activation was also confirmed at the protein level as shown by Western blot, flow cytometry, and immunofluorescence staining. The detection and quantification of single NY-ESO-1 peptides presented on the tumor cell surface in the context of HLA-A*0201 molecules revealed an increase of 100% and 50% for MCF7 and U266 cells, respectively. Moreover, the enhanced expression of NY-ESO-1 derived peptides on the cell surface was accompanied by an increased specific lysis of MCF7 and U266 cells by HLA-A*0201/NY-ESO-1(157-165) peptide specific chimeric antigen receptor (CAR) CD8+ T cells. In addition, the killing activity of CAR T cells correlated with the secretion of higher IFN-gamma level s. These results indicate that NY-ESO-1 directed immunotherapy with specific CAR T cells might benefit from concomitant DAC treatment.

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Resolving RNA-DNA damage-induced genomic instability and cancer: Where there is SETX, there is a way

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Defects in DNA repair have been linked to multiple human genetic disorders. The neurodegenerative disease ataxia with oculomotor apraxia 2 is caused by defects in senataxin (SETX) {1}. This putative RNA/DNA helicase is considered to be an important player in the resolution of RNA/DNA hybrids (R-loops) formed either during transcription termination or the RNA-DNA damage response (RDDR) {2}. Previous studies have shown that SETX localizes to sites of collision between components of the replisome and the transcription apparatus and that it is targeted to R-loops, where it plays an important role at the interface of transcription and RDDR {2,3}. Accumulating evidence indicates that R-loops may be an important source of replication stress-induced tumourigenesis. We find that loss of SETX in both human and mouse cells causes hypersensitivity to treatment with agents that cause either replication stress or induce the formation of R-loops. Furthermore, SETX deficiency promotes the formation of replication stress-induced genomic instability and chromosomal rearrangements. Using genomic approaches, we find that loss of SETX results in altered gene expression, differential methylation patterns and copy number alterations. A combination of chromatin and DNA/RNA immunoprecipitation experiments revealed that SETX deficiency promotes the accumulation of both DNA damage markers and R-loops simultaneously

across many replication stress hotspots, such as regions of the genome that contain transcribing genes, fragile sites and repetitive DNA sequences. The mechanistic links between SETX, replication stress and cancer will be discussed.

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FOXM1 is a critical mediator of DNA damage-induced senescence in gastric cancer models following targeting the MET receptor tyrosine kinase

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The MET receptor tyrosine kinase regulates many signalling pathways involved in proliferation, migration and angiogenesis. Its activity is deregulated in up to 80% of gastric cancers where it correlates with advanced-stage tumors and poor prognosis. In this study, we examined the impact of targeting MET in vitro and in vivo using a highly selective anti-MET small molecule in gastric cancer models. Due to the emerging role of MET in tumor cell responses to DNA damage, we investigated the effects of MET inhibition (METi) combined with ionizing radiation (IR), the most widely used clinical tool of radiation therapy. We report for the first time that METi potentiates the antitumor effect of IR by promoting DNA damage-induced senescence. Mechanistically, we show that METi down-regulates the Forkhead box protein M1 (FOXM1), a key promoter of DNA repair and a prime negative senescence effector, whose activity is largely regulated by the MAPK pathway. Subsequently, FOXM1 ectopic expression was shown to abrogate MET targeting-dependent increase of γH2AX and associated senescence. These data suggest that MET targeting-related senescence results on one hand from accumulation of DNA damage and on the other hand from down-regulation of the major negative senescence program mediator, FoxM1, both events being mediated via METi. The effects of METi are currently under investigation using organotypic cultures established from patient tumor tissues. Our data highlight the potential benefit of using METi in MET-overexpressing tumors to enhance radiotherapy-associated control of tumor growth.

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Bone marrow injury and its consequences on hematopoietic reconstitution following radiation conditioning and hematopoietic cell transplantation

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Allogeneic hematopoietic cell transplantation (HCT) is the only treatment option for many hematological malignancies. Donor T cells are critical for tumor control, but also target host tissues (graft-versus-host; GVH disease). However, little attention is paid to subclinical effects of alloreactive T cells on the bone marrow (BM) and how structural damage affects hematopoiesis. Here, we study the effects of conditioning and allogeneic HCT on BM microarchitecture and hematopoietic reconstitution using flowcytometry and 3D-confocal microscopy. To this point we determined in B6-mice that by day 4 after lethal (950 cGy) and sublethal (475 cGy) total body irradiation (TBI) there were no residual Lin-Sca1+cKit+ hematopoietic stem cells (HSC) detectable; HSC started to recover around day 14 following sublethal TBI. Regarding the non-hematopoietic, stromal compartment on day 4 post lethal and sublethal TBI, there was an increased proportion of endothelial cells (CD45-Ter119-Sca1+CD31+). Morphologically, by day 4 early signs of extracellular matrix destruction were detectable, manifesting as sinusoidal vasodilation; by days 7-10 adipocytes appeared and ultimately dominated the hollow spaces between completely destroyed extracellular matrix and severely dilated sinusoids. In mice given sublethal irradiation, extracellular matrix and sinusoids started to recover and adipocytes disappeared around day 14. These experiments set the basis for currently ongoing studies trans-

planting allogeneic, T-cell-replete grafts. Preliminary data show even more distinct marrow hypocellularity following transplantation of alloreactive T cells than occurred after sublethal irradiation alone. In summary, hematopoiesis is a 3D-process of interactions between hematopoiesis and their microenvironment. Conditioning and GVH-related damage of the stromal microarchitecture influence hematopoietic reconstitution following allogeneic HCT significantly, and should be further investigated.

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Chemotherapy revived – intraarterial cisplatin-based chemotherapy in an osteosarcoma xenograft model

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Osteosarcoma (OS) patients have five-year-survival rates (5-ysr) of approximately 60% after receiving standard systemic intravenous (IV) application of cytotoxic drugs such as cisplatin (CDDP). However, intraarterial (IA) chemotherapy can potentially improve treatment efficacy. Although clinical trials performed earlier failed to show a clear benefit, some studies using IA CDDP demonstrated 5-ysr of >80%. Therefore we evaluated IA chemotherapy under controlled experimental conditions. Intratibial OSs were induced using 143B cells. After tumor establishment (determined by caliper), CDDP (at 4 mg/kg) or vehicle (0.9% NaCl) was administered IV (tail vein) or IA (arteria femoralis). Infusion was performed using custom-made polyethylene catheters and a syringe pump. During the treatment period, blood flows and body weights (BW) were monitored. Post mortem, kidney damage and numbers of metastases were assessed. These studies indicated that IA CDDP yielded the largest reduction of tumor volume measured by caliper (87±20%; mean±SEM % of initial tumor volume) compared to IV CDDP (251±18%), IA NaCl (340±76%) or IV NaCl (426±36%). Furthermore, neither significant loss of BW, nor a significant reduction in blood flow of the tumor leg nor CDDP-specific kidney damage was observed. In addition to tumor volume reduction, IA CDDP significantly reduced the number of micrometastases (IA CDDP: 68±42 (mean±SEM); IV CDDP: 200±15; IA NaCl: 680±296; IV NaCl: 212±41) despite similar numbers of macrometastases . In conclusion, IA infusion of CDDP shows superior efficiency in treating OS xenografts, without increasing kidney damage or loss of BW. Despite being technically challenging, this route of administration should thus be reconsidered for clinical application.

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Nogo-A and S1PR2 as novel regulators of developmental and tumor angiogenesis in the central nervous system

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Glioblastoma are among the most common brain tumors, and are associated with a poor prognosis. One hallmark of glioblastoma growth is angiogenesis,

the formation of new blood vessels. Accordingly, there is a great interest in identifying novel regulators of brain tumor angiogenesis (1). We have identified a new function for the axonal growth-inhibtor Nogo-A as a negative regulator of angiogenesis in the developing central nervous system (CNS) (2). Interestingly, the Nogo-A specific receptor was recently identified as the sphingosine 1-phosphate-receptor 2 (S1PR2)(3). Based on these exciting findings, we investigated a potential role for Nogo-A and S1PR2 in angiogenesis during CNS development and in brain tumors, as well as possible molecular interactions with the VEGF-VEGFR2-Dll4-Jagged-Notch pathway. So far, we could show that during mouse and human brain development, Nogo-A is expressed in the vicinity of growing blood vessels and endothelial tip cells expressing S1PR2 in vivo. At the functional level, the number of endothelial tip cells was significantly increased in Nogo-A-/- as well as in S1PR2-/- mice, indicating a negative regulatory role for this ligand-receptor pair in vivo. During mouse and human glioblastoma angiogenesis, Nogo-A showed a perivascular expression pattern whereas S1PR2 was expressed on the glioblastoma vasculature. In mouse glioblastoma, S1PR2 was upregulated within the glioblastoma as compared to the surrounding brain tissue and in vicinity of brain tumor endothelial tip cells. Moreover, Nogo-A inhibited the adhesion, spreading, migration and filopodia- and lamellipodia of primary murine brain-derived microvascular endothelial cells (MVECs) as well as of human glioblastoma-derived MVECs in vitro. Strikingly, these effects were partly mediated by S1PR2 indicating a negative regulatory role for Nogo-A-S1PR2 in developmental brain- and glioblastoma-angiogenesis.

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Data mining The Cancer Genome Atlas

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The Cancer Genome Atlas (TCGA) has involved a huge collaborative effort to characterize a large number of cancers through multiple platforms which include exome sequencing, comperative genomic hybridization arrays, DNA methylation arrays, RNA sequencing, reverse protein phase arrays, and clinical features (http://cancergenome.nih.gov). The increasing availability of next-generation sequencing tools has enabled basic and clinical researchers to produce and access omics-level data at an unprecedented level like cBioportal [1]. However, most scientists do not have the training to interpret high-dimensional datasets to their maximum potential. To directly address this problem, we have designed a simple web-tool to conduct Kaplan-Meier survival analysis, multivariate Cox Regression analysis, Exome mutation profiling, differential gene expression, pathway analysis, and differential protein analysis on the open source TCGA cancer genome datasets. Our tool is generalizable to any cancer type, is easy to use, and allows for a more precise control of parameters than any other currently available method. This tool acts as a discovery or validation platform for scientists and clinicians without a substantial background in bioinformatics to look at their gene of choice in a specific cancer in terms of gene expression and survival.

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Leucine rich adaptor protein 1-like (LURAP1L), an unknown protein with potential tumor suppressing function in glioblastoma

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Glioblastoma (GBM, WHO grade IV) is the most common and lethal form of glioma. Despite advances in molecular characterization and therapy, prognosis remains very poor with a median survival of 15 months. Our previous study has shown that Wnt inhibitory factor 1 (WIF1) has a tumor suppressor effect in GBM[1]. Gene expression profiles of a WIF1-inducible cell line indicated that LURAP1L is upregulated upon WIF1 induction, thus suggesting a potential tumor suppressing function of this protein. Therefore we investigated the phenotype induced by LURAP1L overexpression and the underlying molecular pathways affected by the protein. GBM cell lines stably overexpressing the LURAP1L protein were established to characterize the phenotype induced. First results confirmed a potential tumor suppressing effect of LURAP1L since it reduced cell proliferation and anchorage-independent growth in soft agar. Moreover, closer analysis of the colony formation assay suggested that LURAP1l overexpression inhibited the propensity of cells to grow on top of each other. Taken together, these results suggest that the protein may affect cell-cell adhesion. Concerning signalling pathways that could be associated with the phenotype observed, we focused on both the canonical (β-catenin) and non-canonical (JNK-AP1) Wnt pathway and NF-κB. Surprisingly both JNK and NF-κB signalling pathways were induced upon Lurap1l overexpression, whereas the canonical Wnt pathway was not affected. Activation of JNK and NF-κB might be due to a feedback loop. The cells may try to block the negative effect of LURAP1L on proliferation by activating NF-κB and JNK pathways.

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Malignant T cells are the major primary immunosuppressive factor in cutaneous T cell lymphoma

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Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of malignancies of skin-homing T cells. The median overall survival for patients with advanced stage IV CTCL is poor. Interestingly, severe systemic infections, such as sepsis, and not malignant T-cell burden itself, are the most common cause of death in these patients. Previously we described how the cytokine microenvironment plays an essential role in cancer immune recognition and survival, and is a key component of the signal transmission through the immunological synapse during infections. Progressive impairment of cellular immunity is a hallmark of CTCL. We could demonstrate that the malignant T cell themselves are a source of extreme excess of suppressive Th2 cytokines, and neutralization of Th2 cytokines and/or reduction of the tumor burden could prevent the global Th2 bias and restore Th1 but not Th17 immune responses. We further hypothesized, and could consequently obtain experimental evidence for an aberrant immune regulatory mechanism in lymphoma on the level of dendritic cells (DC)-T cells interactions, which misshaped the immune response against "non-self" and prevented differentiation of benign effector CD4+ populations towards protective Th1, Th17, and Th22 responses. The underlying mechanisms involved overexpression of inhibitory cell surface molecules on the clonal malignant T cells and loss of information in the immunological DC-T cell synapse. On the level of innate immunity, these studies demonstrate a novel and general immune escape mechanism in cancer that allows cancer cells to evade recognition from the innate immune system, and subsequently abrogate the differentiation of protective anti-tumoral effector CD4+ T cell populations. A translational approach of an immunomodulatory therapy for CTCL that will allow benign blood and tissue resident T cells to outnumber their malignant counterparts may help rebuild protection against infections and even the tumour itself.

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A novel self-lipid antigen targets human T cells against CD1c+ leukaemias

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T lymphocytes that recognize self-lipids presented by CD1c are frequent in the peripheral blood of healthy individuals and kill transformed hematopoietic cells, but little is known about their antigen specificity and potential anti-leukemia effects. We report that CD1c self-reactive T cells recognize a novel class of self-lipids, identified as methyl-lysophosphatidic acids, which are accumulated in leukemia cells. Primary acute myeloid and B-cell acute leukemia blasts express CD1 molecules. Methyl-lysophosphatidic acid-specific T cells efficiently kill CD1c+ acute leukemia cells, poorly recognize non-transformed CD1c-expressing cells, and protect NOD/scid mice against CD1c+ human leukemia cells. The identification of immunogenic self-lipid antigens accumulated in leukemia cells and the observed leukemia control by lipid-specific T cells in vivo provide a new conceptual framework for leukemia immune surveillance and immunotherapy.

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Polyfunctional Th17 infiltrating human colorectal cancers contribute to recruit immune cells associated to favorable prognosis

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Tumor infiltration by immune cells has been recognized to be a key factor determining clinical outcome. Whereas the presence within tumor tissues of defined lymphocytic populations, including cytotoxic CD8+ T cells and IFN-gamma-producing T helper (Th)1 cells, has been unequivocally recognized

to predict favorable prognosis, the clinical relevance and the pathophysiological role of IL-17-producing cells remain unclear. In this study we have investigated clinical relevance and functional features of tumor-infiltrating IL-17-producing cells in human colorectal cancer (CRC). Upon analysis of a tissue micro-array including 1,400 cases of primary CRC, we found that the presence of IL-17+ infiltrates was associated with an early tumor stage, although this factor per se did not impact on overall patient survival. Interestingly, numbers of IL-17+ cells strongly correlated with those of CD8+ and CD16+ myeloperoxidase (MPO)+ neutrophils, which were predictive of better clinical outcome in the same patient cohort. Phenotypic analysis revealed that the majority of tumor infiltrating IL-17+ cells consisted of polyfunctional Th17, producing a spectrum of inflammatory cytokines and chemokines, in addition to IL-17. Tumor-derived Th17 cells promoted migration of neutrophils through IL-8 and enhanced MPO release in vitro. Furthermore, they favored recruitment of CD8+ T cells either by triggering CCL5 and CXCL10 release by tumor-associated endothelial cells or, more surprisingly, by directly targeting CCR5+CCR6+ CD8+ T cells through CCL5 and CCL20 release. Importantly, the direct effect of Th17 proved sufficient to drive CD8+ T cells into an engineered CRC tissue-like structure. Thus, by producing a spectrum of cytokines and chemokines beyond IL-17, Th17 cells promote tumor infiltration by beneficial immune cells, therefore contributing to a more favorable clinical outcome in human colorectal cancer.

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Quantitative (phospho)proteomics on serial tumor biopsies from a sorafenib-treated patient

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Activation of compensatory signaling pathways in tumors confers evasive or intrinsic resistance to targeted therapy, but the pathways and their mechanisms of activation remain largely unknown. To understand mechanisms underlying resistance, we describe a procedure for quantitative proteomics and phosphoproteomics (Super-SILAC) on immediately, snap frozen hepatocellular carcinoma (HCC) and control biopsies from human patients. We applied this procedure to monitor signaling pathways in serial biopsies taken from an HCC patient before and during treatment with the multi-kinase inhibitor sorafenib. Sorafenib was confirmed to inhibit MAPK signaling in the tumor, as measured by reduced Rsk phosphorylation. Hierarchical clustering indicated factors that are up- or down-regulated only in the tumor upon treatment and which may mediate evasive resistance to sorafenib. Enrichment analysis revealed pathways broadly implicated in the development of resistance, such as cell adhesion, further substantiating our findings. Thus, we provide a procedure for quantitative analysis of oncogenic pathways in tumors and how these change in response to treatment. This procedure will allow stratification of patients and elucidation of mechanisms of resistance.

Charles Rodolphe Brupbacher Stiftung

Charles Rodolphe Brupbacher Foundation

Mme Frédérique Brupbacher

Portrait by Peter Cerutti

Charles Rodolphe Brupbacher Stiftung

Die Stiftung hat das Ziel, die Krebsforschung in der Schweiz und international zu fördern.
Wichtigstes Element ihrer Tätigkeit ist die Verleihung des Charles Rodolphe Brupbacher Preises für Krebsforschung, verbunden mit einem wissenschaftlichen Symposium in Zürich.

Die Stifterin

Frau Frédérique Brupbacher hat im November 1991 in Verehrung ihres Gatten, Charles Rodolphe Brupbacher, eine Stiftung mit Sitz in Vaduz errichtet. Die Stiftung verleiht alle zwei Jahre den Charles Rodolphe Brupbacher Preis für Krebsforschung an Wissenschaftler, die in der Grundlagenforschung herausragende Leistungen erbracht haben. Die Preisverleihung findet statt im Rahmen eines internationalen wissenschaftlichen Symposiums.

Auf Antrag der Medizinischen Fakultät ernannte die Universitätsleitung Frau Frédérique Brupbacher 2005 zum Ständigen Ehrengast der Universität Zürich, in Anerkennung der grossen Verdienste, die sie sich mit ihrem Altruismus und ihrem Engagement für die Krebsforschung erworben hat. Durch ihre Initiative und ihren persönlichen Einsatz konnte die Krebsforschung im Raum Zürich nachhaltig gestärkt werden. Am 20. Juni 2001 ernannte Präsident Jacques Chirac sie zum Chevalier de la Légion d'Honneur.

Charles Rodolphe Brupbacher Foundation

The mission of the Foundation is to foster cancer research in Switzerland and internationally.
The key element of its activities is the Charles Rodolphe Brupbacher Prize for Cancer Research which is awarded in association with a scientific symposium in Zurich.

The Founder

In honour of her late husband Charles Rodolphe Brupbacher, Mrs. Frédérique Brupbacher set up a foundation registered in Vaduz, Liechtenstein, in November 1991. The Foundation's mission is to present the biennial Charles Rodolphe Brupbacher Prize for Cancer Research to a scientist with internationally acknowledged meritorious achievements in the field of fundamental research. The Prize is awarded in the context of a scientific symposium.

The Executive Board of the University of Zurich appointed Mrs. Frédérique Brupbacher in 2005 as a permanent Guest of Honor of the University, in appreciation of her altruism and her engagement for 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.

Mitglieder des Stiftungsrates:

Frédérique Brupbacher, Monaco, Präsidentin

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Dr. Lukas S. Keller, Zürich

Prof. Dr. Alexander Knuth, Zürich

Veit de Maddalena, Zürich

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Foundation Board

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

Members of the Foundation Board:

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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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Scientific Advisory Board

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

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