Charles Rodolphe Brupbacher Stiftung

Cancer Genome and DNA Repair

Edited by

Ulrich Hübscher
Gilbert Lenoir
Paul Kleihues

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Charles Rodolphe Brupbacher Preis für Krebsforschung 2011

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 2011 wird verliehen an:

Bert Vogelstein, Baltimore, USA

Jan H. J. Hoeijmakers, Rotterdam, Niederlande

Charles Rodolphe Brupbacher Prize for Cancer Research 2011

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 2011 Prize are:

Bert Vogelstein, Baltimore, USA

Jan H. J. Hoeijmakers, Rotterdam, The Netherlands

Charles Rodolphe Brupbacher Preis für Krebsforschung

Universitätsspital Zürich, Grosser Hörsaal Nord, Frauenklinikstrasse 10, 8091 Zürich
Donnerstag, 17. Februar 2011, um 17:00 Uhr

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

Felix Mendelssohn, Klaviertrio Nr. 1 in d-Moll Op. 49, Molto allegro agitato

Laudationes
Prof. Dr. Bert Vogelstein
Prof. Dr. Jan Hoeijmakers

durch

Prof. Dr. Paul Kleihues
Prof. Dr. Charles Weissmann

Preisverleihung
Frau Frédérique Brupbacher, Präsidentin der Stiftung

Franz Liszt, Liebestraum Nr. 3 in As-Dur Op. 62, Poco allegro con affetto

Referate der Preisträger
Prof. Dr. Bert Vogelstein

Camille Saint-Saëns, Le Carnaval des Animaux Nr. 13, Der Schwan

Prof. Dr. Jan Hoeijmakers

Sergej W. Rachmaninov, Vocalise Nr. 14 Op 34, Transkription für Klaviertrio

Schlussworte
Prof. Dr. Klaus W. Grätz

Apéro

Charles Rodolphe Brupbacher Prize for Cancer Research

University Hospital Zurich, Lecture Hall Nord, Frauenklinikstrasse 10, 8091 Zurich
Thursday, February 17th, 2011, 17:00 pm

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

Felix Mendelssohn, Piano trio Nr. 1 in d-Moll Op. 49, Molto allegro agitato

Laudationes
Prof. Dr. Bert Vogelstein
Prof. Dr. Jan Hoeijmakers

by

Prof. Dr. Paul Kleihues
Prof. Dr. Charles Weissmann

Awards
Mrs. Frédérique Brupbacher, President of the Foundation

Franz Liszt, Liebestraum Nr. 3 in As-Dur Op. 62, Poco allegro con affetto

Acceptance Speeches
Prof. Dr. Bert Vogelstein

Camille Saint-Saëns, Le Carnaval des Animaux Nr. 13, The Swan

Prof. Dr. Jan Hoeijmakers

Sergej W. Rachmaninov, Vocalise Nr. 14 Op 34, Transkription for piano trio

Final address
Prof. Dr. Klaus W. Grätz

Apéro

Charles Rodolphe Brupbacher Foundation

The
Charles Rodolphe Brupbacher Prize
for Cancer Research 2011
is awarded to

Dr. Bert Vogelstein

for his contributions to

The genetic basis of cancer, in particular the
sequential acquitistion of genetical alterations
during colon carcinogenesis

The President
of the Foundation

Frédérique Brupbacher

The President
of the Scientific Advisory Board

Prof. Dr. Klaus W. Grätz

Laudatio

Paul Kleihues

The human genome is present in every cell of our body. Whenever a cell divides, the DNA must be duplicated and this happens with remarkable speed and precision. The faithful synthesis of two new DNA strands takes just 6-8 hours and the entire process of cell division about 20 hours. However, to err is human and this also applies to DNA replication. Spontaneously, or induced by environmental carcinogens, all kinds of error may occur, including point mutations, insertions, deletions and gene amplifications. If not corrected by the DNA repair machinery, disease, including cancer may result.

Today, we honour Dr. Bert Vogelstein, a physician scientist who has made groundbreaking discoveries on the genetic basis of human cancers. He was particularly interested in identifying genetic alterations that initiate the development of early neoplastic lesions, and which subsequently cause a progression to malignant, metastasizing carcinomas. Rather than concentrating on animal models, he chose to investigate carcinoma of the colon, a common human cancer closely associated with the Western life style. Choice of this organ site was very wise since colon cancer typically develops over a long period of time, from a microadenoma to a small, then larger adenoma, to early carcinoma and ultimately to a highly malignant metastasizing carcinoma. Dr. Vogelstein showed that each of these steps is caused by the acquisition of additional genetic alterations. The earliest detectable change is a mutational inactivation of the APC/beta-catenin pathway, followed by an oncogenic activation of K-RAS which is typically present in small adenomas. Clonal expansion with progression to carcinoma is then caused by subsequent activation of other oncogenes and inactivation of the tumour suppressor gene p53. About 17 years must pass for a large benign adenoma to evolve into an advanced cancer, but then less than 2 further years are needed to acquire the ability to metastasize. Elucidation of this time frame and identification of the associated genetic changes, have enormous implications for cancer prevention and the development of novel diagnostic and therapeutic strategies. Dr. Vogelstein's genetic model of colorectal tumorigenesis, initially published in 1988 and 1990 has been cited more than 10'000 times by authors worldwide. It has opened the door to the understanding that phenotypic changes observed clinically and histologically

during tumour progression reflect the sequential acquisition of genetic alterations. Graphic displays showing this for other tumour types have deferentially been called 'vogelgrams'.

Bert Vogelstein and colleagues showed that mutational inactivation of the TP53 suppressor gene plays an important role in more than 50% of all human cancers, and that it acts as DNA-binding protein. His laboratory identified the APC gene as a causative factor in the development of hereditary adenomatous polyposis and, together with Albert de la Chapelle's group in Helsinki, that deficient DNA mismatch repair causes hereditary non-polyposis colorectal cancer.

Sometimes, it takes time for a discovery to acquire clinical significance. In 1993, Dr. Vogelstein's group identified the zinc finger protein GLI that is up-regulated in medulloblastomas, the most common and most malignant of pediatric brain tumours. GLI mediates hedgehog signaling and has recently been shown to be effectively inhibited by therapeutic arsenic.

Much of Dr. Vogelstein's success is based on the development of new analytical tools. With his collaborators Victor Velculescu and Kenneth Kinzler, he developed a novel technique for the serial analysis of gene expression (SAGE), which allows the identification and quantitation of genes that are expressed in specific cell types.

More recently, Dr. Vogelstein's laboratory embraced new DNA sequencing tools that allow screening of the entire cancer genome. The sequence analysis of glioblastomas revealed a plethora of gene alterations previously unknown to contribute to the development of this most common and malignant of brain tumour. They discovered frequent mutation of the isocitric dehydrogenase gene IDH1/2 in astrocytomas and oligodendrogliomas. This mutation occurs with a remarkable lineage specificity: it is present in secondary glioblastomas that develop from low-grade astrocytomas, but not in primary de novo glioblastomas, indicating an origin of these subtypes from different glial precursor cells.

Dr. Vogelstein is probably the most frequently cited author in the entire field of biomedical research. Over more than two decades he has been a pioneer in uncovering the genetic basis of cancer which is a prerequisite for novel targeted therapies.

Many cancer scientists have been dancing to his music, both metaphorically and literally. At the annual reception of the American Association for Cancer Research, he and his colleagues' band 'The Wild Type' played pure, non-mutated rock music.

We are all delighted that today Bert Vogelstein joins the prestigious list of distinguished scientists who have been awarded the Charles Rodolphe Brupbacher Cancer Prize.

Dr. Bert Vogelstein

Summary Curicculum vitae

Date of Birth	June 2, 1949
Current Position	Professor of Oncology Professor of Pathology The Johns Hopkins University School of Medicine and Investigator, Howard Hughes Medical Institute
Location	The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Room 589 1650 Orleans Street Baltimore, Maryland

Qualifications

1966 – 70	B.A., Mathematics University of Pennsylvania, Philadelphia
1970 – 74	M.D. The Johns Hopkins University School of Medicine

Previous appointments

1974 – 75	Pediatric Internship. The Johns Hopkins Hospital
1975 – 76	Pediatric Residency. The Johns Hopkins Hospital
1976 – 78	Research Associate. National Cancer Institute
1978 – 83	Assistant Professor, Oncology. The Johns Hopkins Uni School of Medicine
1983 – 89	Associate Professor, Oncology. The Johns Hopkins Uni School of Medicine
1989 –	Professor, Oncology.The Johns Hopkins Uni School of Medicine
1992 –	Joint Appointment in Molecular Biology and Genetics. The Johns Hopkins Uni School of Medicine
1995 –	Investigator. Howard Hughes Medical Institute
1998 –	Professor, Pathology. The Johns Hopkins Uni School of Medicine
2005 –	Co-Director, Ludwig Center for Cancer Genetics & Therapeutics. The Johns Hopkins Uni School of Medicine

Selected Honours and Awards

1976	American Academy of Pediatrics - Maryland Medical Society Research Award
1989	Rhoads Memorial Award, American Association for Cancer Research, for „achievements in cancer research“
1990	Bristol-Myers Squibb Award, Distinguished Achievement Cancer Research
1992	Distinguished Alumnus Award, Johns Hopkins University
1992	Herman Beerman Lecture, Society for Investigative Dermatology
1992	Young Investigator Award, American Federation for Clinical Research
1992	American Cancer Society Medal of Honor
1992	Election to National Academy of Sciences, USA
1993	American Cancer Society Research Professorship
1993	Shacknai Memorial Prize, Hebrew University, Jerusalem for „outstanding research in cancer biology“
1993	Pezcoller Foundation Award, European School of Oncology, for „outstanding contributions to oncology research“
1994	Howard Taylor Ricketts Award, University of Chicago for „outstanding accomplishments in medical science“
1994	Ernst Schering Prize, Ernst Schering Research Foundation, for „extraordinarily outstanding work in basic research“
1995	G.H.A. Clowes Memorial Award, American Assn. for Cancer Research,
1995	David A. Karnofsky Memorial Award, American Soc for Clinical Oncology
1996	Cancer, AIDS, & Immunology Research Institute Award, Bar-Ilan University
1997	Golden Plate Award, American Academy of Achievement
1997	Award for Excellence in Medicine, American-Italian Cancer Foundation
1998	Paul Ehrlich and Ludwig Darmstaedter Prize, Paul Ehrlich Foundation
1998	William Allan Award, American Society of Human Genetics,
1999	Alfred G. Knudson Award, National Cancer Institute
1999	Outstanding Research Award, American Society of Clinical Pathologists
2000	Charles S. Mott Prize, General Motors Cancer Research Foundation
2001	International Chiron Award, Italian National Academy of Medicine
2001	Harvey Prize in Human Health,The Tech nion, Haifa
2002	Am Assoc of Pathologists Award for Excellence in Molecular Diagnostics
2004	Prince of Asturias Award in Science, “for research that represents a significant contribution to the progress of humanity”, Prince of Asturias Foundation, Oviedo, Spain
2006	New York Academy of Medicine Medal for Distinguished Contributions to Biomedical Science

2006	Election as Fellow to American Association for Advancement of Science
2007	Pioneer in Science Award, American Research Forum
2008	Pasarow Award for Extraordinary Accomplishment in Medical Research, Robert J. and Claire Pasarow Foundation
2009	Massachusetts General Hospital Award for Cancer Research
2009	Science of Oncology Award, American Society of Clinical Oncology

Editorial Appointments

1988 – 90	Cancer Research, Associate Editor
1988 –	Genes, Chromosomes, and Cancer, Associate Editor
1988	Science, Board of Reviewing Editors
1993 –	Cancer and Metastasis Reviews, Editorial Board
1994 – 2001	New England Journal of Medicine, Editorial Board
1998 –	Molecular Cell, Associate Editor
2001 – 2005	Nature Reviews Cancer, Advisory Panel
2002 –	Cancer Cell, Associate Editor
2002 –	Cell Cycle, Editorial Board
2002 –	Cancer Biology and Therapy, Editorial Board

Selected Reviews

1. Fearon, E.R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell 61: 759-767, 1990.
2. Kinzler, K.W., and Vogelstein, B. Lessons from hereditary colorectal cancer. Cell 87: 159-170, 1996.
3. Lengauer, C., Kinzler, K.W., and Vogelstein, B. Genetic instabilities in human cancers. Nature 396: 643 – 649, 1998.
4. Yan, H., Kinzler, K.W., and Vogelstein, B. Genetic testing – present and future. Science 289: 1890-1892, 2000.
5. Vogelstein, B., Lane, D., and Levine, A.J. Surfing the p53 network. Nature 408: 307-310, 2000.
6. Vogelstein, B. and Kinzler, K.W. Cancer genes and the pathways they control. Nature Medicine 10: 789-799, 2004.

Publications

As of 2010, four hundred and twenty two (422) papers in professional journals including
Nature, Science, New England Journal of Medicine Nature Reviews Cancer Cell, The Lancet, Cancer Cell Nature Medicine, Science Translational Medicine, Proceedings of the National Academy of Sciences, U.S.A. Nature Biotechnology, Cancer Research, Oncogene Journal of the National Cancer Institute

The Genetic Basis of Human Cancer and Its Implications for Patient Management

Bert Vogelstein

Forty years ago, cancer was a mysterious disease, with numerous theories invoked to explain its occurrence. Some thought it was caused by the breakdown of immunity as individuals age. Others believed that infectious agents such as viruses and bacteria were the major culprits. Another group of scientists hypothesized that it was caused by aberrant genes. And a competing theory posited that the genes in cancer cells were normal, but that they were expressed aberrantly.

As a result of world-wide efforts to understand the molecular basis of cancer since that time, cancer is no longer mysterious. We now know that cancer is, in essence, a genetic disease. In other words, it is driven by alterations in DNA that change the sequence or regulation of the genes that normally control cell birth and cell death - oncogenes and tumor suppressor genes, respectively. Oncogenes are like the accelerators in an automobile; a mutation in an oncogene is akin to having the pedal stuck to the floor. Tumor suppressor genes function like the brakes in an auto - a cell without its normal tumor suppressor genes is akin to an auto without brakes, impossible to control. In normal cells, the ratio between cell birth and cell death is exactly 1.00. If cell death exceeds cell birth, tissue atrophy results. If cell birth exceeds cell death because an oncogene or tumor suppressor gene is mutated, then a tumor results.

Cancers are notably different from other types of genetic diseases, such as muscular dystrophy or cystic fibrosis. Most diseases considered to be genetic are caused by inherited mutations. In cancers, the mutations are not generally inherited from the parents. Instead, most mutations develop after birth and occur in isolated, individual cells rather than in every cell in the body. A second major difference is that no single mutation „causes“ cancer. A sequential series of mutations is required for a clinically significant cancer to occur, each one leading to a greater ratio of cell birth to cell death.
Colorectal cancers provide an excellent example of the principles enumerated above. A colorectal tumor is initiated when a single, normal colorectal epithelial cell acquires a mutation in either the APC or β-catenin gene. This mutant cell gradually forms a microscopic tumor because its ratio of cell birth to cell death is higher than that of its surrounding non-mutant normal epithelial cells. One of the progeny of the mutant clone may eventually acquire a second mutation, such as one in the KRAS or BRAF genes. This now doubly mutant cell has an even greater selective advantage and can grow to a macroscopically visible size - a benign tumor called a polyp or adenoma. A third mutation, then a fourth, fifth, etc., in genes such as PIK3CA, p53, SMAD4, MRE11, FBXW7, occur by chance in successive generations. The resulting avalanche of mutations leads to tumor progression, culminating in invasive growth and metastasis from the primary site in the colon to the liver, lung, and other organs.

Colorectal cancers also illustrate another important concept relating genes to tumorigenesis. A small fraction of patients who develop colorectal cancers have a strong family history of the disease. There are two major forms of such hereditary predisposition, one called Familial Adenomatous Polpyposis (FAP) and the other called Hereditary Non-polyposis Colorectal Cancer (HNPCC). Patients with FAP inherit a mutant form of the APC gene and therefore develop hundreds to thousands of small polyps. But no single mutation is sufficient to engender cancer - it requires multiple mutations, as outlined above. Because there are so many polyps in FAP patients, however, at least one of them is likely to gradually acquire other mutations in KRAS, p53, PIK3CA, etc. and eventually progress to cancer.

HNPCC develops through a different route. These patients inherit a mutant form of an enzyme involved in repairing mistakes during DNA replication, a sort of spell-checking mechanism. HNPCC patients do not develop multiple polyps, but instead develop only one or a few, at the same frequency as the general population. But once a polyp is formed, the defect in repairing mistakes is revealed and the tumors acquire mutations in other genes - including oncogenes and tumor suppressor genes - in a relatively rapid fashion, so that tumorigenesis is accelerated. FAP can therefore be thought of as a disease of tumor initiation while HNPCC is a disease of tumor progression. Both initiation and tumor progression are required for a fully mature, lethal cancer to develop.

The last few years have witnessed further great strides in understanding the cancer genome. It has become possible to precisely identify the entire compendium of genes that are altered in a cancer. These studies have shown that colorectal cancers - like those of other common tumors such as breast, pancreas, and brain - have an average of ~70 gene-altering mutations. Roughly ten of these are in oncogenes or tumor suppressor genes, the others are „passengers" that have coincidentally occurred in the same cell that acquired a mutation in a „driver" cancer gene. Any two colorectal cancers, even though they may look the same under the microscope, have a different, overlapping set of mutations. This genetic variation explains much of the heterogeneity in the biologic properties of tumors and the differential responses of cancers to chemotherapeutic drugs and radiation.

Now that the basic landscape of the cancer genome is known, one of our major challenges is to figure out how to use this information to reduce morbidity and mortality from neoplasia. New therapies that target the mutant genes or the pathways through which these genes operate are being aggressively pursued in both industry and academia. But there is another route to achieving this goal that I believe will, in the final analysis, be equally important. Detailed studies of the temporal development of colorectal and pancreatic cancers have shown that it takes a long time - decades in fact - for a cell to accumulate all the mutations required to make it malignant. It is only in the last two or three years of this thirty-year journey that the tumor acquires the ability to travel outside its primary site, that is, to metastasize. And it is only in this last few years - once the tumor has metastasized - that a patient cannot be cured of his or her cancer through surgery alone. In essence, nearly every patient who dies from colorectal, pancreatic, or other solid tumors does so only because the presence of a cancer was not detected for the first twenty or thirty years of its existence.

The flip side of this observation is that the long development time provides a large window of opportunity for detecting incipient cancers while they are still curable by conventional surgical procedures. The new studies on the genomic landscapes of cancer are providing new opportunities for such early diagnosis. These opportunities include molecular imaging techniques that detect the abnormal pathways present in cancer cells through radioactive isotopes or non-radioactive probes. These abnormal pathways are also associated with changes in protein composition of the cancer cell, and some of these proteins leak into the circulation where they can in principle be detected by novel blood tests. A third opportunity involves detection of the mutant oncogenes and tumor suppressor genes themselves. These mutant sequences are the best possible biomarkers available; they are not simply associated with cancer - they are the proximate cause of cancer. Members of my laboratory have been attempting to detect these mutant genes in plasma and other bodily fluids in patients with cancer. Though these studies are still ongoing, it is already clear that circulating mutant genes can be detected in the great majority of patients with advanced disease and a significant fraction of patients with early-stage cancer.

The history of medicine shows that once a disease is understood, it is only a matter of time before suffering from that disease diminishes. That same history shows that for most diseases, the breakthroughs come from prevention rather than cure. In 50 years, cancer will not be the problem that it is today, in part because of improvements in therapy but even moreso because most cancers will either be prevented entirely or detected at a relatively early stage, when they can be managed by simply removing them with a scalpel.

Charles Rodolphe Brupbacher Foundation

The
Charles Rodolphe Brupbacher Prize
for Cancer Research 2011
is awarded to

Dr. Jan H. Hoeijmakers

for his contributions to

The elucidation of the molecular basis of
inherited DNA repair deficiencies and
their role in cancer and aging

The President
of the Foundation

Frédérique Brupbacher

The President
of the Scientific Advisory Board

Prof. Dr. Klaus W. Grätz

Laudatio

Charles Weissmann

I have come to realize that for a professor it is more rewarding to educate students than to write papers, because the half-life of papers is about three years while that of students is 30 years. Therefore, aging professors like to tot up their successful students, and may lay claim to mentorship with only marginal justification. Thus it came about that when Paul Kleihues mentioned to me that Jan Hoeijmakers was the awardee of the Brupbacher Prize 2011, I proudly announced that he had been my student in Zurich, maybe neglecting to mention that this was only for a month or two. In response, I was promptly asked to present the laudatio, providing me with the much appreciated opportunity of once again attending a Brupbacher Symposium and meeting many friends, former colleagues and last but not least, Jan.

In 1979 Jan was a student of my brilliant former collaborator Piet Borst, working at Amsterdam University, and it was essential for his project to characterize the genes encoding trypanosome surface antigen VSG. At that time the Dutch considered recombinant DNA technology as being so dangerous an activity that it was virtually interdicted in the Netherlands. In Switzerland we had managed to implement more reasonable working rules and as a consequence my erstwhile laboratory at the University of Zurich was well positioned to carry out gene cloning. Thus, Jan came to our lab with his RNA preparations and after some amusing setbacks was successful in obtaining the desired clones. This allowed him to show that trypanosomes can switch VSG expression by DNA rearrangement and resulted in a notable Nature paper. After moving to Dirk Bootsma's lab in Rotterdam he participated in the first cDNA cloning of ERCC1, a human DNA repair enzyme, and followed this up by identifying a plethora of human genes underlying nucleotide excision, postreplication and recombination repair, including the first genes implicated in the DNA repair syndromes xeroderma pigmentosum, Cockayne Syndrome and Trichothiodystrophy. Xeroderma pigmentosum is of particular interest in the context of this symposium: the various forms of this disease are caused by deficiency of one of the several enzymes involved in Nucleotide Excision Repair, and lead to an enhanced mutation rate. If tumor suppressor genes, such as p53, are affected, an increased frequency of skin cancers may result. Jan generated a collection of mouse mutants mimicking the defects of human Nucleotide Excision Repair diseases which not only proved valuable for elucidating the role of DNA damage and repair on carcinogenesis but

also uncovered a link to the aging process and to the biological clock. Thus, repair-deficient mice may exhibit premature aging, with a life-span as short as 3 weeks, and a complete inactivation of the circadian rhythm. Another important outcome of Jan's research, in collaboration with Jean-Marc Egly, was that the perplexing combination of clinical symptoms caused by Cockayne Syndrome and Trichodystrophia came about because some repair proteins are components of the transcription factor TFIIH and in their absence basal transcription is impaired. Interestingly, the shortened life span goes hand in hand with decreased cancer incidence.

Jan is not only an outstanding scientist, but he is admired for his generosity towards colleagues and competitors. He has collaborated with many groups, thereby promoting the field as a whole. Jan's insights have led him to search for practical approaches to retard the aging process and to found a company with this aim.

We rejoice that Jan's seminal contributions are being rewarded with the Charles Rodolphe Brupbacher Prize for Cancer Research 2011.

Jan H. Hoeijmakers

Summary Curicculum vitae

Date of birth	March 15, 1951
Current Position	Professor of Molecular Genetics, Faculty of Medicine, Erasmus University
Location	Dept of Genetics Erasmus Medical Center, PO BOX 2040, 3000 CA Rotterdam, The Netherlands,

Qualifications

B.Sc.	Biology, Catholic University of Nijmegen, The Netherlands, 1973
M.Sc.	Biology, specialization Biochemistry (Genetics, Chemical Cytology, Biochemistry), Catholic University of Nijmegen, 1975 (cum laude)
Ph.D.	Section Medical Enzymology and Molecular Biology, Lab. of Biochemistry, University of Amsterdam, The Netherlands, 1982 (promotor Prof. Dr. P. Borst)

Previous Appointments

Graduate student:	Laboratory of Biochemistry University of Amsterdam, The Netherlands, 1975 – 1979
Research Fellow:	Department of Microbiology, University of Amsterdam, The Netherlands, 1979 – 1981
Senior Scientist:	Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands, 1981 – 1985
Associate Professor:	Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands, 1985 – 1993
Prof Molecular Genetics:	Department of Cell Biology and Genetics, Erasmus Univesity, Rotterdam, The Netherlands, 1993 – present.

Honours and Awards

- ‚Harold Quintus Bosz' Prize (Utrecht, 1983, for the discovery of the molecular mechanism of antigenic variation in trypanosomes, PhD thesis)
- ‚Snoo van t' Hoogerhuys' Prize (Utrecht, 1986, for isolation of the first human DNA repair gene)
- The very prestigious ‚Louis Jeantet' Prize for Medical Research in Europe (Geneva, 1995, for the work on DNA repair, shared with D. Bootsma).
- 'Spinoza' Prize for research by the Dutch Organization for Scientific Research (NWO, Den Haag, 1999)
- 'Descartes-Huygens' Award for French-Dutch scientific collaborations (2000) (French Science Organization).
- • 'Van Gogh' Award from the Dutch Science Organization (2000)
- "EC-Descartes" Award for European collaboration on DNA repair syndromes by the European Community (shared with: Egly, Lehmann and Stefanini) (Brussels, 2000)
- 'Josephine Nefkens' Award for cancer research (Rotterdam, 2001, for fundamental contributions to cancer research)
- Seneca Medaille des Industrie-Clubs für Altensforschung Prize, (Düsseldorf, 2008) for pioneering research on the molecular basis of aging.
- ERC Advanced Grant-Life Sciences (2008) (European Research Council, ~2 M€ in the domain of multi-disciplinary sciences, on the basis of the entire scientific oeuvre)
- Cancer Research Prize of the Charles Rudolph Brupbacher Stiftung, (Zurich, 2011) (for research on the role of genome stability on cancer and aging, together with Bert Vogelstein)

In addition elected member of KNAW (section 'geneeskunde, department 'natuurkunde', 2000), elected member of EMBO (1995).

Editorial appointments

- Carcinogenesis (till 1993 – 1999)
- J. Cell Science (till 1992 –1 998)
- Mutation Research (1989 – 2002)
- Experimental Cell Research (1994 – 2000)
- Arch. Biochem. Biophys. (1997 – present)
- Genes and Development (1997 – 2002)
- EMBO J. (1997 – 2002)
- EMBO reports (2000 – 2005)
- Cancer Cell (2002 – present)
- DNA repair (2002 –present)

(Co)organiser of international meetings – selected listing

- International Workshop on DNA Repair (Noordwijkerhout NL, 1988)
- EC Concerted Action Meeting on DNA repair and Cancer (Oxford, 1998)
- Juan March Workshop on: 'The interface between transcription, repair, recombination and chromatinremodelling' (Madrid, 1999)
- International Workshop on DNA repair (Noordwijkerhout NL, 2001)
- „DNA Repair and Mutagenesis: From Molecular Structure to Biological Consequences", Fairmont Southampton, Princess, Bermuda, December 7-13, 2003.
- 19th annual Forbeck Forum on Cancer, Hilton Head, USA, November, 6-9, 2003

- Molecular Basis of Aging, Titisee Conference. Boehringer Ingelheim Foundation. (Titisee 2007)
- AACR, Genetic Instability Mechanisms, (Los Angeles, USA, 2007)
- International Scientific Meeting on Aging CBG/CGC (Amsterdam, NL 2011)

Patents

- Prematurely ageing mouse models for the role of DNA damage in ageing and intervention in ageing-related pathology. Patent Office: Vereenigde Octrooibureau. Year: 2007. Patent number: EP1815245. On this patent the spin-off company DNage is based.
- Compositions for prevention and treatment of DNA damage and ageing syndromes. Patent Office: Vereenigde Octrooibureau. Year: 2007. Patent number: WO2007133076. Follow-up patent in the context of DNage
- Detection methods based on HR23 protein binding molecules. Patent Office: Vereenigde Octrooibureau. Year: 2004. Patent number: WO2004046683. Patent intended to enable reduction of animal testing.
- Screening assays for compounds that cause apoptosis. Patent Office: Townsend. Year: 2004. Patent number: US2004115747
- Human RAD. Patent Office: GIMMI. Year: 1997. Patent number: WO9742209.
- Several patents are in preparation in the area of healthy aging.

Publications

As of 2010, three hundred and fifty (350) papers in professional journals including Nature, Science, Nature Cell Biology, Nature Genetics, Cell, Cancer Cell, Molecular Cell, Aging Cell Genes and Development, Molecular Cell Biology, Proceedings of the National Academy of Sciences, U.S.A. Cancer Research, American Journal of Human Genetics, ENBO Journal, Current Opinion in Cell Biology, Nucleic Acids Research.

DNA damage and its impact on cancer, aging and longevity

J.H.J. Hoeijmakers, G. Garinisa, B. Schumacherb, J. Pothof, I. van der Pluijmc, J. Mitchell, H. van Steegd, and G.T.J. van der Horst. Genetics, Erasmus MC, PO Box 2040, 3000 CA Rotterdam, a) Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece; b) Univ. of Cologne, Cologne, Germany; c) DNage, Leiden; d) RIVM, Bilthoven, The Netherlands

DNA, the carrier of genetic information, is incessantly damaged by exogenous agents (UV-, gamma-radiation and numerous natural or man-made chemicals), which are in part avoidable and -importantly- also by inevitable (by)products of normal cellular metabolism. The latter include reactive oxygen and nitrogen species and other natural reactive cellular metabolites. DNA damage may have two major, distinct consequences. Some DNA lesions induce permanent changes in the genetic code (mutations) upon replication or chromosomal aberrations after mitosis. Alternatively, DNA injury may trigger permanent cell cycle arrest or cell death. To counteract the negative effects of damage to our genes a complex genome maintenance apparatus has evolved comprised of an intricate network of DNA repair systems and cell cycle checkpoints which acts as a guardian of the genome. Each repair system is designed to eliminate a specific category of DNA damage. For instance, nucleotide excision repair (NER) removes a wide range of helix-distorting lesions of exogenous origin (UV-induced lesions, bulky chemical adducts), but also endogenous damage (e.g. oxidative cyclopurines). The molecular mechanism of NER involves >30 proteins acting in a multi-step reaction: lesion detection, local opening of the DNA helix, lesion verification and stabilization of the open NER intermediate, dual incision of the damaged strand to release the lesion as part of a 22-30 oligonucleotide, gap-filling DNA synthesis and final ligation to the pre-existing strand. Two NER sub-pathways exist. Global genome (GG-)NER covers the entire genome and is particularly important for preventing mutations. Transcription-coupled repair (TCR) removes damage that obstructs ongoing transcription to enable recovery of this vital cellular process and mainly counteracts cytotoxic effects of DNA injury.
Several rare, autosomal recessive inherited NER syndromes are known which are characterized by extreme sun(UV)sensitivity,

but otherwise display a striking clinical heterogeneity: very strong (skin)cancer predisposition in xeroderma pigmentosum (XP) as well as dramatic neuro-developmental deficits as in Cockayne syndrome (CS) and the brittle hair disease trichothiodystrophy (TTD). Remarkably, although life expectancy in the latter 2 conditions is frequently limited to childhood, they appear not associated with any cancer susceptibility, in striking contrast to XP. Intriguingly, mutations in core NER helicases XPB and XPD, which are subunits of the repair/transcription factor TFIIH, are associated with all three disorders or combinations.

To assess the medical impact of DNA damage and NER and to get insight into the puzzling clinical heterogeneity we have generated a series of transgenic mouse mutants, several with identical mutations in NER genes as found in NER patients. E.g. XPDTTD mice, mimicking an XPD point mutation of a TTD patient exhibit strikingly similar clinical features as the human syndrome including the characteristic brittle hair. Detailed analysis of these mice revealed that TTD is in fact a segmental premature aging syndrome, like CS, which is indeed less susceptible to spontaneous cancer. XPDXP/CS mutant mice, on the other hand, carrying a XPD point mutation of a patient with combined XP and CS, are highly predisposed to cancer, but also display premature aging, demonstrating that both phenotypes can also co-exist. Different single and double NER mutants exhibit multiple premature aging features, including osteoporosis, neuro-degeneration, early infertility and cessation of growth, liver and kidney aging, deafness, retinal photoreceptor loss, depletion of hematopoietic stem cells, etc. Life span is limited to ~1,5 year for milder mutants to 3-5 weeks for dramatic double mutants. A striking correlation is found between severity and type of compromised repair and rate of onset and severity of the clinical aging manifestations providing strong experimental support for the DNA damage theory of aging. The different defects in DNA repair and their effects on cancer and aging can be rationalized as follows. Generally, compromised GG-NER, which eliminates distorting DNA injury over the entire genome, leads to enhanced damage levels everywhere, which -upon replication- will increase mutagenesis and thereby cancer. TCR only focuses on lesions in the transcribed strand of active genes that arrest transcription. Since this is only a very small proportion of all DNA damage in the genome this repair system has little impact on mutagenesis and cancer, but is vital for resumption of transcription and thereby for cellular viability. Defects in TCR will thus render cells more sensitive to DNA damage-induced cell death, thereby strongly protecting from cancer, but at the expense of enhanced cell death which in turn accelerates aging. GG-NER mutations in TCR/GG-NER double mutant mice will enhance the overall DNA damage load, which aggravates the TCR problems causing even earlier cell death further reducing life expectancy. Conditional mutants in which specific dramatic aging occurs only in e.g. the brain, display many signs of neurodegeneration whereas the remainder of the body appears normal, revealing organ-specific accelerated aging. We propose that endogenous lesions hamper transcription and replication triggering cellular apoptosis-senescence and in the end (premature) aging.

Microarray, functional and physiological studies have revealed that persisting DNA damage elicits a systemic downregulation of the IGF1 somatotrophic axis and upregulation of anti-oxidant defences, favouring maintenance and defences at the expense of growth and development, explaining the severe growth defect of repair mutants and progeroid NER patients. Persisting DNA damage triggers this 'survival' response in a cell autonomous manner and implicates regulation by a set of ageing-related microRNAs. Caloric restriction and fasting trigger a similar 'survival' response, which maximizes anti-oxidant defence and -when constitutive- promotes longevity at least under laboratory conditions. These data link accumulation of DNA damage and the IGF1 control of life span and open perspectives for the promotion of healthy aging, including reduced risk of cancer.

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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 Immunityand 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»

Programm des Wissenschaftlichen Symposiums 2011

Program of the Scientific Symposium 2011

Wednesday, February 16, 2011

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

13:00 - 15:00 **Welcome: Ulrich Hübscher / Gilbert Lenoir**

Personalized treatment and resistance
Chair: Gilbert Lenoir

Michael O. Hottiger, Zürich
PARP inhibition: more than just synthetic lethality?

Anne-Lise Børresen-Dale, Oslo
Towards personalized treatment for breast cancer

Michael Weller, Zürich
Novel treatment approaches to glioblastoma

Georgia Chenevix-Trench, Brisbane
Pharmacogenetics and personalized medicine in ovarian cancer

15:00 - 15:30 *Coffee break*

15:30 - 17:30 **Cancer epigenetics**
Chair: Adrian Bird

Susan M. Gasser, Basel
Role of nucleosome remodelers in DNA repair: Novel players in cell survival

Peter A. Jones, Los Angeles
The nucleosome, the epicenter of the epigenome

Adrian Bird, Edinburgh
The dinucleotide CpG as a genomic signalling module

Robyn L. Ward, Kensington, Sydney
Transmission of epigenetic mutations in humans with cancer

17:30 - 18:30 *Apéro*

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

Harald zur Hausen, Heidelberg
Krebsprävention durch Impfung

Thursday, February 17, 2011

08:00 - 08:30 *Registration*

08:30 - 10:00 **DNA repair and cancer genome (1)**
Chair: Ulrich Hübscher

Grigory L. Dianov, Oxford
Base excision repair targets for cancer therapy

Josef Jiricny, Zürich
Identification of KIAA1018/FAN1 and its role in interstrand cross-link repair

Fred W. Alt, Boston
High throughput cloning of the B lymphocyte translocatome

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

11:00 - 12:00 **DNA repair and cancer genome (2)**
Chair: Piet Borst

Bert Vogelstein, Baltimore
Cancer genomes and their implications for research and patients

Jan H. Hoeijmakers, Rotterdam
DNA damage and its impact on cancer, aging and longevity

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

14:00 - 15:00 **DNA repair and cancer genome (3)**
Chair: Susan M. Gasser

Michael R. Stratton, Cambridge
Evolution of the cancer genome

Stephen C. West, South Mimms
Genes involved in the maintenance of genomic stability

15:00 - 16:00 **Immunotherapy (1)**
Chair: Alexander Knuth

Robert D. Schreiber, St. Louis
Cancer immunoediting: immunologic control and shaping of cancer

Cornelis J. M. Melief, Leiden
Cancer immunotherapy by dendritic cells

16:00 - 17:00 *Break*

17:00 - 18:30 **Award ceremony**
Charles Rodolphe Brupbacher Prize for Cancer Research 2011

18:30 - 19:30 *Apéro*

Friday, February 18, 2011

08:00 - 09:00 **Immunotherapy (2)**

Thomas F. Gajewski, Chicago
Profiling of metastatic melanoma to identify strategies to overcome immune resistance in the tumor microenvironment

Wolf Hervé Fridman, Paris
Integrating immunology with cancer biology and medicine

09:00 - 10:00 **Cancer stem cells (1)**
Chair: Lukas Sommer

Andreas Trumpp, Heidelberg
Dormancy in normal and malignant stem cells

Cédric Blanpain, Bruxelles
Epithelial stem cells during DNA damage and cancer initiation

10:00 - 10:30 *Coffee break*

10:30 - 11:30 **Cancer stem cells (2)**
Chair: Klaus Rajewsky

Jean-Pierre Bourquin, Zürich
Leukemia initiating cells are frequent and oligoclonal in de novo resistant acute lymphoblastic leukemia

Jeffrey M. Rosen, Houston
Intrinsic therapeutic resistance of breast cancer stem cells

11:30 - 12:30 **Young Investigator Awards**
Referees: Piet Borst, Robert D. Schreiber, Bernard W. Stewart, Stephen C. West
Awards presented by Mme. F. Brupbacher

Abstracts
Eingeladene Redner

Abstracts
Invited Speakers

Krebsprävention durch Impfung

Harald zur Hausen, Heidelberg

Nobelpreis Medizin 2008

Innerhalb der vergangenen 30 Jahre konnten etwa 21% der globalen Krebsinzidenz mit infektiösen Ereignissen in Verbindung gebracht werden. Dies betrifft spezifische virale, bakterielle und parasitäre Erreger. Insbesondere hat die Entdeckung der Rolle von Hepatitis B Virus beim Leberzellkrebs und von Hochrisiko-Papillomviren (HPV) bei Gebärmutterhalskrebs, anderen Krebserkrankungen des Anogenital- und Mundhöhlen-Bereichs neue Ansätze der Krebsprävention durch Impfung ermöglicht. Würden diese Impfstoffe global für den entsprechenden Personenkreis eingesetzt, könnten sie theoretisch das Krebsrisiko für Frauen um 12-14% reduzieren, für Männer nur etwa um 4-5%. Wenig erfolgreich waren bisher Versuche, weitere Impfstoffe gegen zusätzliche Krebsviren zu entwickeln. Spezifisch für Hepatitis C und humane Immundefizienz Virus (HIV) Infektionen hat die Impfstoffentwicklung nicht zu signifikanten Erfolgen geführt. Dies beruht wesentlich auf intragenomischen Veränderungen der entsprechenden Virus-Nukleinsäuren, die früh nach der Primärinfektion auftreten. Ebenso wenig hat der Einsatz therapeutischer Impfstoffe bisher zu überzeugenden Erfolgen geführt.
Es werden zusätzlich Überlegungen vorgetragen, ob weitere weit verbreitete Krebserkrankungen des Menschen, die zurzeit nicht mit Infektionen in Zusammenhang gebracht werden, dennoch mit infektiösen Ereignissen in Verbindung stehen könnten. Dies betrifft zum Teil Krebserkrankungen, die vermehrt unter Immunsuppression auftreten, aber auch solche, deren Inzidenz unter Immunsuppression nicht erhöht – oder sogar reduziert ist. Auch maligne Tumoren, die auf der Basis anderer, primär nicht transformierender Infektionen entstehen, sollen diskutiert werden. Einige Infektionen verdienen besondere Erwähnung, da sie offensichtlich das Risiko für spezifische nachfolgende Krebserkrankungen reduzieren (akut lymphatische Leukämien und andere Krebserkrankungen des blutbildenden Systems) Schließlich sollen noch potenzielle synergistische Effekte von Ernährungsfaktoren mit Virusinfektionen analysiert werden. Der Nachweis einer solchen Beziehung sollte in Zukunft zu neuen Konzepten in Krebsprävention und Krebstherapie führen.

Ausgewählte Literatur:

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PARP inhibition: more than just synthetic lethality?

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ADP-ribosylation is a post-translational modification of proteins, catalyzed by ADP-ribosyltransferases (ARTs) that utilize NAD^+ as a substrate. Subsequent elongation of the protein-bound ADP-ribose moiety generates poly-ADP-ribosylated proteins. The family of cellular ARTs, which are similar to the first structurally characterized ADP-ribosyltransferase diphtheria toxin (ART diphtheria toxin-like (ARTD)), comprises 18 proteins. The best-studied ARTD family member is poly-ADP-ribose polymerase 1 (PARP1; now renamed ARTD1). PARP1/ARTD1 is an abundant chromatin-associated nuclear protein, which acts to ADP-ribosylate proteins, including itself, thus affecting their physiological functions.

Inhibitors of PARPs/ARTDs are emerging as a promising new class of anticancer agents particularly effective against breast and ovarian tumors harboring germline or somatic defects in DNA repair and DNA damage signaling genes (i.e., ATM, BRCA1, BRCA2, NBS1 or PTEN). This reflects a new strategy of drug development known as „synthetic lethality" in which two genes are said to be in a synthetic lethal relationship if a mutation in either gene alone is not lethal but mutations in both cause the death of a cell. Exciting data from ongoing clinical trials strengthen this concept showing that PARP inhibitors are active as single agents to tumor patients with carrier mutations in *BRCA1* and *BRCA2* genes.

Although historically studied in the context of DNA damage detection and repair, PARP1/ARTD1 and its enzymatic activity has more recently been linked to the regulation of chromatin structure, compaction and chromosome organization. Our laboratory provided evidence that PARP1/ARTD1 directly modifies histone proteins at distinct lysine residues know to be modified also by other enzymes (e.g. histone acetyltransferases). Additional results support the notion that PARP1/ARTD1-dependent ADP-ribosylation regulates chromatin structure as well as function (e.g. for transcription or DNA synthesis). These findings provide new insights into the role of cellular ADP-ribosylation as well as PARP1/ARTD1's physiological function and potentially expand future directions for PARP inhibition in the clinical setting.

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Towards Personalized treatment for breast cancer

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Breast cancer is a major public health problem and the incidence is increasing, particularly among younger women. Recent progress in screening programs, diagnosis and postoperative adjuvant systemic therapies has resulted in increased survival, but still close to 25% of the patients experience relapse and die from metastatic disease. The assessment of risk profiles in early presentation of breast disease is predominantly based on X-ray mammography, clinical examination, histopathological evaluation of tumor biopsies and on lymph node status, which is the strongest prognostic factor. However, the obtained information is often not sufficient for accurate risk stratification or predicting response to treatment, particularly with regards to evaluating the potential for drug resistance. Breast cancer is a complex disease caused by accumulation of genetic alterations leading to a disturbance in the balance between proliferation and apoptosis, genetic instability and acquisition of an invasive and resistant phenotype. The inherent heterogeneity that characterizes all malignant tumors, in addition to the diverse contribution from the

normal microenvironment, will contribute to each tumors phenotype and will dictate the molecular portrait of the tumors. Currently, only a handful of clinical, pathologic and molecular factors help clinicians decide upon diagnosis, prognosis and selection of therapy. The heterogeneity of breast cancer is only partially apprehended by these parameters, making therapeutic strategies less than perfectly adapted to each patient. In addition, current cytotoxic drugs do not differentiate between malignant and normal cells. Thus, developing molecular therapies selectively targeting the tumor cells, and also adapted to each individual patient, are highly prioritized areas in the future. Microarray and sequencing technologies, applied to the study of DNA/mRNA/miRNA, can be used to portray a tumor's detailed phenotype in its unique context, and to generate molecular signatures that will improve our understanding of the causes and progression of the disease, for the discovery of new molecular markers, for therapeutic intervention and for developing new prevention strategies. We have performed such analyses of more than 1000 breast carcinomas of different stages and histological types aiming at novel tumour classification that can predict survival and treatment response. By integrating data from the patient's own genotype with data from the tumor at the DNA level, (copynumber, mutations, methylation), mRNA and miRNA level, as well as metabolic profiles revealed from HR-MAS MR analyses of the tumor, we seek to reach a more fundamental understanding of the biological dynamics of breast cancer. This will facilitate identification of risk factors, search for novel cancer diagnostics, prediction of therapeutic effects and prognosis and identification of new targets for therapy.

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Novel treatment approaches to glioblastoma

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Glioblastoma is the most common and most malignant primary brain tumor and is rated grade IV in the World Health Organization (WHO) classification of brain tumors. It has traditionally been viewed as the final stage after malignant progression from lower grade glial tumors, including diffuse astrocytomas and oligodendrogliomas (WHO grade II) and their anaplastic variants (WHO grade III). However, very recent molecular and clinical observations have demonstrated that glioblastoma is a distinct entity most often unrelated to other glial tumors. Mutations of p53 and the isocitrate dehydrogenase (IDH) 1/2 genes are much more common in gliomas of WHO grades II and III whereas amplification of the epidermal growth factor receptor (EGFR) and phosphatase-and-tensin homolog (PTEN) genes are more common in glioblastoma. For decades, neurosurgical resection, whenever possible, and postoperative radiotherapy have remained the cornerstones of treatment for gliomas. More recently, alkylating chemotherapy using temozolomide has been defined as a novel standard of care when combined with radiotherapy in newly diagnosed glioblastoma. In contrast, temozolomide alone has become an alternative to radiotherapy alone in patients with grade II and III gliomas. Methylation of the promoter region of the O6-methylguanylmethyltransferase (MGMT) gene has emerged as a potent predictor of benefit from temozolomide chemotherapy in glioblastoma patients, but not in patients with grade III gliomas who appear to benefit equally from chemotherapy or radiotherapy if their tumor exhibits MGMT promoter methylation. Conversely, the outcome of patients with low-grade gliomas, who are treated by surgery alone, does not correlate with the molecular signatures defined by either p53 mutations, 1p/19q codeletion, MGMT promoter methylation or IDH mutations. Altogether, there has thus been made significant progress in molecular neuropathology, and the determination of a set of molecular markers will soon be implemented in the diagnosis and clinical decision making for patients with gliomas.

Beyond classical approaches of DNA damaging treatments such as radiotherapy and chemotherapy, the most promising current therapeutic approaches target angiogenesis, an essential requirement for tumor growth that is particularly characteristic of glioblastoma. Competing agents include bevacizumab, an antibody to vascular endothelial-derived growth factor (VEGF), cediranib, a VEGF receptor blocker, cilengitide, an integrin antagonist, and enzastaurin, a protein kinase C-β antagonist. The phase III registration trial exploring cilengitide is unique in that it limits patient accrual to a molecularly defined subtype of glioblastoma, that is, glioblastoma with MGMT promoter methylation. This decision was based on the results of a phase II trial which suggested preferential benefit from cilengitide in that patient population and presents another step towards personalized cancer therapy in neuro-oncology.

In addition to these antiangiogenic approaches, which have already reached the stage of development of phase III clinical trials, the most promising further experimental treatment approaches include the targeting of tumor-specific metabolic pathways and various modes of vaccine-based immunotherapy. These novel strategies commonly seek to target specifically the (hypothetical) population of cancer stem cells which are thought to be the origin of the tumor, to maintain viability even during multimodality treatment, and to ultimate cause relapse, progression and death.

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Pharmacogenetics and personalized medicine in ovarian cancer

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Most ovarian cancer patients undergo surgery, followed by chemotherapy with paclitaxel and carboplatin. Although initially effective in the majority of patients, tumour response in individual patients can vary widely and the clinical course can be difficult to predict. A few patients with advanced ovarian cancer will be cured, the largest group will respond initially but eventually relapse with drug-resistant disease, and up to 30% will progress while on first-line chemotherapy. The ability to predict drug response could identify patients who are now exposed to the toxic effects of chemotherapy without significant benefit, influence optimal dosing and allow selection of a potentially more effective chemotherapy regime.

We performed a multi-stage genome-wide association study (GWAS) for drug response in ovarian cancer. In our first stage we used 385 patients from the Australian Ovarian Cancer Study (AOCS), the Mayo Clinic and the The Cancer Genome Atlas (TCGA) project. All patients had been treated with paclitaxel/carboplatin and we evaluated Progression Free Survival as the primary outcome measure. We maximized power and limited costs by genotyping cases with extreme phenotypes from the AOCS, taking into account the extent of residual disease following surgery. For the AOCS data, restricting analysis to a defined, homogeneous treatment subgroup of 142 cases, treated with a minimum of four cycles of Carboplatin AUC 5 or 6 and Paclitaxel 135 or 175 mg/m2, revealed strong evidence (p=2.0e-6, rs1211152) for association at the previously identified *ABCB1 (MDR1)* locus (1), but this effect was diminished in analysis of the full AOCS data set of 183 cases which included cases for which the dose was unknown. We are currently following up the top findings from our GWAS in a large independent replication cohort of ~1500 treated cases from seven studies within the Ovarian Cancer Association Consortium, and these results will be presented.

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Role of nucleosome remodelers in DNA repair: Novel players in cell survival

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Interphase chromatin is intrinsically mobile, but little is known about the forces that move DNA, nor about the functional impact of chromatin mobility on nuclear processes. We have examined the molecular components involved in chromatin mobility. First we tested whether chromatin movement is dependent on transcription, the Gal4 activation domain (GAD), the strong activator VP16, or components of the remodeling factor INO80. We exploited high precision live fluorescent microscopy in budding yeast to quantitatively assess chromosome mobility. We found that unlike the local recruitment of Gcn5, Rpd3 or GAD, both VP16 and INO80 components significantly increase the diffusion coefficient of a locus, as well as the number of large steps, as well as the radius of constraint of two independent genomic loci. Importantly, recruiting LexA-Ino80^{K737A}, an ATPase dead mutant, to a genomic locus did not increase mobility. Increased chromatin mobility through Ino80 targeting increased the spontaneous frequency of repair by homologous recombination (HR), which is dependent on a homology search step. We have also examined possible roles of the SWR1-C complex and one its actin-related subunit, Arp6, both of which are recruited to DSBs. We found that Arp6 binds some promoters and telomeres in a Swr1-independent manner, and can shift chromatin to the nuclear envelope independently of Swr1 or H2A.Z deposition, unlike Arp5 or Arp8, which are components of the INO80 complex.

We recently showed in budding yeast that irreparable breaks are recruited to the nuclear periphery, whereas physiologically relevant spontaneous breaks occurring during S-phase preferred an internal location. We sought to define the mechanisms that distinguish different types of DNA damage and regulate chromatin movement during repair. We used fluorescence microscopy to visualize and quantify DSBs location and movement in living cells. We

find that all spontaneous breaks marked by functional fusions of YFP with Mre11, Rad52, Rad51, or Rad54 are internal, providing strong evidence that HR repair of DSBs occurs in the lumen and not the nuclear envelope. In addition, the movement of Mre11-repair foci are as fast as an intact chromatin locus, whereas later in the process, when Rad52 is recruited, repair foci become highly constrained despite their internal localization. Interestingly, both Rad51 and Rad4 are required for internal localization and for constrained movement of Rad52-YFP foci. This was not affected by inhibition of the checkpoint kinases. We are now testing the hypothesis that chromatin movement is controlled by remodelling of chromatin at sites of DNA breaks and that chromatin movement may contribute to homology search.

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The Nucleosome, the Epicenter of the Epigenome

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The nucleosome is the fundamental building block of chromatin and is the integrator of epigenetic information. The 146 bp of DNA which is subject to cytosine methylation, is in close contact with a histone octamer containing histone monomers subject to covalent modification which convey epigenetic information. Nucleosomes are inherently refractory to transcription initiation and have to be moved or evicted to expose DNA and make it available for the transcription apparatus. We have designed a new methodology to map nucleosomal distribution by using the enzymes SssI (1) or M.CviPI (2) which methylate cytosine residues in the CpG or GpC context respectively. We then extract DNA from treated nuclei and clone it after bisulfite treatment to get a single molecule view of the distribution of nucleosomes (3). This approach coupled with chromatin immunoprecipitation gives us an extraordinarily precise view of chromatin structure. We have used this method to show that nucleosomes slide to cover the transcription start site during mitosis (4), that enhancers contain nucleosome depleted regions which can explain the abilities of transcription factors to reprogram cells and have shown that nucleosomal occupancy precedes DNA methylation at the Oct4 and Nanog promoters. We also show that the DNMT3a and 3b are firmly anchored to nucleosomes and cosediment with them on sucrose density gradients (5). Knowledge of these processes is fundamental to understanding the degeneration of aberrant DNA methylation patterns during carcinogenesis.

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The Dinucleotide CpG as a genomic signalling module

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The DNA sequence 5'CG (CpG) is unusual in several respects. It is self-complementary and can exist in three forms depending on the modification status of its cytosine moiety. The local density of CpG also varies dramatically within genomic DNA. Throughout most of the mammalian genome it is rare and highly methylated, but this global methylation pattern is interrupted by short regions where CpG is approximately ten times more abundant than elsewhere. These so-called "CpG islands" (or CGIs) are usually non-methylated and often include the transcription start sites and promoters of genes. By analysing all CGIs in human and mouse tissues we established that more than half are remote from annotated promoters, being intra- or inter-genic. Like promoter CGIs, "orphans" are associated with trimethylation of histone H3 lysine 4 and usually co-localise with RNA polymerase II. CGIs that become methylated during development are almost invariably orphan CGIs, whereas promoter CGIs rarely acquire methylation. In colorectal cancers, on the other hand, gene promoter CGIs frequently become methylated. We propose that orphans mark promoters of as yet uncharacterised transcripts, in particular non-coding RNAs. To understand the functional significance of CGIs, we studied proteins that bind either to CpG (e.g. Cfp1) or to m5CpG (e.g. MeCP2) leading to downstream effects on chromatin structure and gene expression. Our results indicate that these proteins are mediators that allow the underlying sequence of CGIs to influence chromatin modification status. We propose that the DNA sequence at CGIs simplifies genome function by influencing chromatin structure directly.

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Transmission of epigenetic mutations in humans with cancer

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DNA sequence variations and epigenetic modifications which change the activity of genes can determine susceptibility to diseases including cancer. It has long been known that germline sequence mutations in tumour suppressor genes cause cancer predisposition syndromes. Recently, constitutional epimutations have been shown to cause hereditary cancer syndromes such as hereditary non-polyposis colorectal cancer (HNPCC). In this context an epimutation is defined as a change in the epigenetic state of a gene which leads to inappropriate activation or silencing of the gene. In the case of colorectal cancer, constitutional epimutations in the mismatch repair genes *MLH1* (1) and *MSH2* (2) manifest as soma-wide monoallelic promoter methylation and transcriptional silencing. Individuals with constitutional epimutations in the mismatch repair genes are predisposed to the development of young onset microsatellite unstable colorectal, uterine and other cancers. The origins of epimutations are still unknown however evidence exists to support the hypothesis that 'epigenetic predisposition' may occur as a consequence of *cis*- or *trans*-acting genetic sequence variations. Studies of families with epimutations causing diseases such as chronic lymphocytic leukaemia (3) and colorectal cancer have shown that epimutations have occurred secondary to *cis* changes. Despite these observations there are other families with epimutations where no underlying changes in *cis* have been identified.

In these families it is likely that *trans*-acting factors may cause epimutations through their interaction with specific alleles and within particular cellular contexts. An alternate hypothesis is that epimutations and the associated repressive histone modifications are transmitted intact in a proportion of gametes, ('gametic epigenetic inheritance'). There is as yet no evidence for this type of inheritance in humans. Irrespective of origin and mechanism of inheritance, the identification of epimutations in humans informs risk assessment for cancer and other diseases. It also aids the design and application of new therapies which target the epigenome.

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Base excision repair targets for cancer therapy

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Base excision repair (BER) is a frontline DNA repair system that is responsible for maintaining genome integrity, thus preventing many human diseases, including premature aging and cancer, by repairing DNA base lesions and single strand breaks caused by endogenous and exogenous mutagens. It is also the principal DNA repair system in cancer cells that counteracts the killing effect of chemotherapy (alkylating agents) and ionizing radiation (about 80 % of DNA damage induced by ionizing radiation are base lesions), that are the major cancer treatments. Changes in BER capacity are most probably responsible for the variability in the efficiency of treatment of many cases of cancer, since many cancers have altered expression of BER proteins. Although BER enzymes have been studied in detail, the mechanisms involved in BER coordination and regulation of BER capacity are unclear. This knowledge gap is impeding the finding of new cancer therapy targets and the development of novel treatment strategies.

Recently, we have pioneered new studies on the regulation of BER protein levels and activity by post-translational modifications, including ubiquitylation, deubiquitylation and phosphorylation. These studies have included identification of the enzymes involved in regulation of the key BER proteins: DNA polymerase, XRCC1, DNA ligase III and AP-endonuclease. The results of these studies have allowed us to identify the major proteins involved in BER regulation, as well as to formulate novel principles of BER regulation and evaluate their potential as new targets for cancer therapy.

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Identification of KIAA1018/FAN1 and its role in interstrand cross-link repair.

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The mismatch repair (MMR) system has evolved to correct errors of DNA replication and to prevent illegitimate recombination events. However, genetic experiments in several different organisms implicate MMR proteins also in DNA damage signaling, somatic hypermutation and interstrand cross-link (ICL) processing (1). Unfortunately, the genetic data tell us little about the molecular roles of the MMR proteins in these distinct pathways of DNA metabolism.

We carried out a series of pull-down experiments, using human cell lines stably expressing tagged MMR proteins, and characterised the interactome of MLH1 and PMS2 by proteomic analysis. The strongest interactions were verified by reciprocal pull-down experiments and by western blots. Interestingly, some of the principal interactors were proteins involved in the processing of ICLs. This was very puzzling, given that MMR-deficient cells do not generally display markedly different sensitivity to cross-linking agents such as cisplatin and mitomycin C and that cells defective in ICL processing are not known to have a mutator phenotype. We are currently attempting to characterize the role of MLH1 and PMS2 in the metabolisms of ICLs.

Interestingly, one of the interacting partners of MLH1, encoded by a gene named KIAA1018, turned out to be a nuclease that protects cells from ICL-generating agents. This enzyme associates with the *Fanconi anemia* D2/I heterodimer, which recruits it to arrested replication forks, where it presumably cleaves the cross-linked substrate to release the block. We named the protein FANCD2-associated nuclease, FAN1 (2).

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High throughput cloning of the B lymphocyte translocatome

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Recurrent chromosomal translocations and other chromosomal abnormalities are strongly associated with leukemias, lymphomas and some solid tumors. For translocations to occur, two distinct chromosomal loci must be broken, brought together (synapsed), and joined. Subsequently, translocations can clonally appear in tumors in the context of oncogenic selection. We will discuss new insights into the processes and pathways that mechanistically influence the appearance of translocations in lymphoid cells. The presentation will cover the V(D)J or IgH class switch recombination pathways that can initiate DNA double strand breaks (DSBs), the influence of spatial proximity in the interphase nucleus on ability of two DSBs to translocate, and the potential roles of DNA DSB response and DSB end-joining pathways, including alternative end-joining, in suppressing or promoting translocations. A particular focus of the talk will be a description of progress made in elucidating mechanistic factors that promote translocations in B lymphocyte tumors or their progenitors via the development and application of novel high-throughput methods for identifying genome-wide translocation partners („the translocatome") from fixed chromosomal DSBs. We will also discuss new insights into potential roles of long range immunoglobulin or T cell receptor locus regulatory regions and enhancers in promoting expression of translocated cellular oncogenes and, thereby, contributing to their frequent appearance in tumors.

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Cancer genomes and their implications for research and patients

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Human cancer is, in essence, a genetic disease. Though this pivotal fact has been established through work performed in numerous laboratories over the past three decades, the past five years have witnessed a development that would have seemed like science fiction only a short time ago: the ability to identify all somatic alterations affecting all coding genes within a cancer genome. As of this writing (Nov 1, 2010), there have been 84 cancers whose genes have been entirely sequenced, either through whole exome or whole genome approaches. As a result of advances in massively parallel sequencing, thousands of additional tumors will be analyzed in comprehensive fashion in the coming years. Already, however, the landscape of cancer genomes is becoming clear and the implications of this research are evident.

These sequencing data provide answers to basic questions about the number and nature of the somatic mutations in typical solid tumors. For example, the number of coding sequence alterations in adult tumors typically ranges from 30 to 80. Pediatric tumors and leukemias contain considerably fewer mutations (5 to 15 mutations per tumor). The great majority (93%) of alterations in all tumors are single base substitutions, and of these, 82% result in missense changes, 6.5% result in stop codons, and 4.3% result in alterations of splice sites or untranslated regions immediately adjacent to the start and stop codons. A large excess of C to T transitions at 5'-CpG-3' is found in all tumors except breast cancers, which had a unique spectrum.
Subtle mutations such as base substitutions and small insertions and deletions are of course not the only type of genetic alteration in tumors. Among the most important of the other alterations are homozygous deletions and amplifications. The former are associated with the biallelic inactivation of tumor suppressor genes, the latter with oncogene activation. Most adult tumors have one or two homozygous deletions resulting in inactivation of a tumor suppressor gene, and ~40% have amplifications resulting in more than 10 copies of an oncogene per cell.

These data have provided first picture of the genomic landscapes of common solid tumors. They are characterized by a handful of commonly mutated gene "mountains" and a much larger number of gene "hills" that are mutated at low frequency. The data also present several challenges that will occupy cancer researchers in the coming decades. These challenges include:
1. Discrimination between the small hills that represent drivers from those composed of passengers. Driver mutations are causally involved in the neoplastic process and confer a selective growth advantage during some stage of tumorigenesis. Passenger mutations provide no selective advantage but are retained by chance during repeated rounds of cell division and clonal expansion. Mutation frequency alone cannot distinguish between drivers and passengers, and more sophisticated approaches are needed.

2. Analysis of the pathways through which these genes operate. The complexity of cancer genomes can be reduced by realizing that pathways rather than individual genes govern the course of tumorigenesis. Mutations in any of several genes of a single pathway can thereby cause equivalent increases in net cell proliferation. Accordingly, we and others have devised methods to determine whether the genes within specific pathways were mutated more often than predicted by chance. We were thus able to identify 12 processes or pathways that were each genetically altered in the majority of pancreatic cancers analyzed. These pathways and processes were commonly altered in other tumor types, though the particular genes and mutations varied among the tumor types. Further understanding of these pathways, particularly in human cells, will be essential for basic research as well as for drug development.

3. Exploitation of the knowledge to reduce morbidity and mortality from the disease. The explosion in genetic information about tumors has obvious implications for developing targeted therapies. At the same time, the complexity and heterogeneity of cancer genomes raises significant obstacles for such therapy. Another way to reduce morbidity and mortality from cancer is through early detection. Recent molecular genetic studies have confirmed that it requires two or three decades for cancers to form, and it is only in the

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DNA damage and its impact on cancer, aging and longevity

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DNA, the carrier of genetic information, is incessantly damaged by exogenous agents (UV-, gamma-radiation and numerous natural or man-made chemicals) and by products of normal cellular metabolism, including reactive oxygen species. To counteract the negative effects of damage to our genes, a complex genome maintenance apparatus has evolved comprised of an intricate network of DNA repair systems and cell cycle checkpoints. Nucleotide excision repair (NER) is one of the most versatile repair systems removing a wide range of helix-distorting lesions of exogenous (UV, bulky adducts), but also endogenous (e.g. cyclopurines) origin. Two NER sub-pathways exist. Global genome NER covers the entire genome and is important for preventing mutations. Transcription-coupled repair removes damage that obstructs transcription, mainly counteracting cytotoxic effects of DNA injury. Photo(sun)sensitive inherited NER syndromes display a striking clinical heterogeneity: very strong (skin)cancer predisposition in xeroderma pigmentosum (XP) as well as dramatic neuro-developmental deficits as in Cockayne syndrome (CS) and trichothiodystrophy (TTD) remarkably without any cancer susceptibility.

Mutations in NER helicases XPB and XPD, subunits of the repair/transcription factor TFIIH, are associated with all three disorders or combinations. XPD^{TTD} mice, mimicking a XPD point mutation of a TTD patient, revealed that TTD is in fact a segmental premature aging syndrome, like CS, which appears to be protected from spontaneous cancer. $XPD^{XP/CS}$ mutant mice on the other hand are highly predisposed to cancer, but also display premature aging, demonstrating that both phenotypes can co-exist. Different single and double NER mutants exhibit multiple premature aging features, including osteoporosis, neuro-degeneration, early infertility and cessation of growth, liver and kidney aging, deafness, retinal photoreceptor loss, depletion of hematopoietic stem cells, etc. Life span is limited to 1,5 year for milder mutants to 3-5 weeks for dramatic double mutants. A striking correlation is found between severity of compromised repair and rate of onset and severity of

the clinical aging manifestations providing strong experimental support for the DNA damage theory of aging. Conditional mutants in which dramatic aging occurs only in e.g. the brain, display many signs of neurodegeneration whereas the remainder of the body appears normal, revealing organ-specific accelerated aging. We propose that endogenous oxidative lesions hamper transcription/replication and trigger cellular apoptosis-senescence and in the end (premature) aging. Microarray, functional and physiological studies have revealed that persisting DNA damage elicits a systemic downregulation of the IGF1 somatotrophic axis and upregulation of anti-oxidant defences, favouring maintenance and defences at the expense of growth and development, explaining the severe growth defect of repair mutants. Persisting DNA damage triggers this 'survival' response in a cell autonomous manner and implicates regulation by a set of ageing-related microRNAs. Caloric restriction and fasting trigger a similar 'survival' response, which maximizes antioxidant defence and -when constitutive- promotes longevity at least under laboratory conditions. These data link accumulation of DNA damage and the IGF1 control of life span and open perspectives for the promotion of healthy aging, including reduced risk of cancer.

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Evolution of the Cancer Genome

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All cancers carry somatically acquired changes in their genomes. Some, termed "driver" mutations, are causally implicated in cancer development. The remainder are "passengers", and bear the imprints of mutational processes operative during cancer development. Following the advent of second generation sequencing technologies the provision of whole cancer genome sequences has become a reality. These sequences generate comprehensive catalogues of somatic mutations, including point mutations, rearrangements and copy number changes and provide insights into the evolutionary processes underlying the development of individual human cancers, including the factors generating variation and the forces of selection. These insights will form the foundation of our understanding of cancer causation, prevention and treatment in the future.

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Genes involved in the maintenance of genome stability

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Inheritable breast cancers have been linked with mutations in the *BRCA1, BRCA2* and *PALB2* genes, all of which affect the efficiency of double-strand break repair by homologous recombination. Affinity tagged human BRCA2 was expressed in HeLa cells and showed strong interactions with PALB2 and the RAD51 recombinase. Using tandem affinity purification procedures, the BRCA2 protein was purified to homogeneity and found to bind selectively to single-stranded DNA (ssDNA), and to ssDNA regions in tailed duplexes and replication fork structures. BRCA2 protein has been visualized by electron microscopy, and shown to form monomeric and dimeric species, both in solution and with DNA. We find that the BRCA2 tumour suppressor directs the binding of RAD51 to ssDNA, while reducing its ability to bind duplex DNA. As a consequence of this targeted binding, BRCA2 stimulates RAD51-mediated DNA strand exchange reactions. These observations provide a molecular basis for the role of BRCA2 in the maintenance of genome stability.

We are also interested in the mechanisms by which recombination intermediates (Holliday junctions or HJs) are resolved. Human cells possess a variety of HJ processing activities that are essential for chromosome stability. One activity, the BLM protein, inactivated in individuals with Bloom's syndrome (BS), acts in combination with Topoisomerase IIIα, RMI1 and RMI2 to promote HJ dissolution. Cells derived from individuals with BS exhibit an elevated frequency of sister chromatid exchanges (SCEs) indicating that BLM is responsible for the avoidance of crossovers between sister chromatids. We have examined the contribution of HJ resolving nucleases (e.g. MUS81-EME1, SLX1-SLX4 and/or GEN1) to SCE formation in BS cells, and have generated human cells compromised for all known HJ dissolution/resolution pathways. We find that loss of MUS81 or SLX4 reduces SCE formation in BS cells, suggesting that these nucleases promote SCE formation and drive the chromosome instability that underpins the early onset cancers associated with Bloom's syndrome. However, during the course of these studies, we found that inactivation of GEN1 in BS cells lacking either MUS81 or SLX4, resulted in severe chromosome abnormalities. Metaphase spreads from these cells revealed that sister chromatids remained interlinked in a side-by-side arrangement, and the chromosomes were elongated and segmented. Our results indicate that normally replicating cells require HJ processing activities to prevent sister chromatid entanglements, thereby ensuring accurate chromosome condensation.

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Cancer Immunoediting: Immunologic Control and Shaping of Cancer

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Cancer Immunoediting is the process by which the immune system controls and shapes cancer. We originally envisaged that cancer immunoediting would occur in three phases: Elimination (also known as cancer immunosurveillance, the host protective phase of the process), Equilibrium (the phase in which tumor cells that survive immune elimination remain under immunologic growth control resulting in a state of functional tumor dormancy) and Escape (the phase where clinically apparent tumors emerge because immune sculpting of the tumor cells has produced variants that display either reduced immunogenicity or enhanced immunosuppressive activity). Strong experimental data has now been obtained using mouse models of cancer to demonstrate the existence of each phase of the cancer immunoediting process and compelling clinical data suggests that a similar process may also occur during the evolution of certain types of human cancer. Our efforts now focus on elucidating the molecular and cellular mechanisms that underlie each phase of cancer immunoediting and identifying the critical checkpoints that regulate progression from one phase of the process to the next. This approach has helped identify the nature of antigens seen by immunity in nascent developing cancers and has further shown that immunoselection is a major mechanism of immunoediting. Moreover, we have found that edited tumors can still be controlled by the immune system if natural mechanisms that prevent autoimmunity are suspended. As reported by others, we have confirmed that inhibition of CTLA-4 induces ejection of edited MCA sarcomas. However, we have also found that inhibition of PD-L1 does the same, although by perhaps different mechanisms. These differences will be discussed.

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Cancer immunotherapy by dendritic cells

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Therapeutic vaccination of persistent virus infections and associated diseases including (pre-)cancer has largely been without success thusfar, mainly due to the fact that insufficiently consistent and robust effector T cell responses were induced by the commonly used vaccine constructs and formulations, such as recombinant viruses and bacteria, recombinant proteins, DNA constructs, dendritic cell (DC) vaccines and exact HLA class I binding peptides (short peptides). Problems have been severe antigenic competion from vector sequences by recombinant viruses and bacteria, insufficiently powerful T cell generation by DNA constructs, insufficient homing to lymph nodes injected DC and antigen presentation of short peptides by non-professional antigen presenting cells in vivo, causing tolerance instead of immunity. Much more robust and consistent T cell responses can be obtained by vaccination with long (28-35 amino acid long) synthetic peptides. Such immunogens are more efficiently processed and presented than intact proteins by DC and only DC can efficiently perform this task. Moreover only concentrated antigen of choice is offered and antigenic competition therefore plays no role.

In earlier work we showed that therapeutic vaccination with a synthetic long peptide (SLP®) vaccine mediated the eradication of established human papilloma virus type 16 (HPV16)-positive tumors in mice and controlled wart growth and latent virus infection in rabbits persistently infected with cottontail rabbit papilloma virus. Subsequent phase I/II studies with an HPV16 SLP® vaccine, consisting of 13 long peptides covering the HPV16 E6 and E7 antigens, in patients with advanced HPV16-positive cervical cancer, revealed that this vaccine was safe and highly immunogenic. We then tested the clinical efficacy of this HPV16 SLP® vaccine in HPV16-induced high grade vulvar intraepithelial neoplasia (VIN3), a premalignant epithelial disorder, spontaneous regression of which occurs in less than 2% of patients and in which recurrence after standard treatment is high.

In a phase 2 trial, 20 women with VIN3 were vaccinated three times sc in the limbs with a mix of the HPV16 E6 and E7 synthetic long peptides formulated in Montanide ISA-51. The endpoints were objective clinical responses,

defined as reduction of at least 50% in lesion size (partial response) or complete regressions, and HPV16-specific T-cell responses.
The vaccine was safe. At 3 and 12 months after the last vaccination an objective response was observed in 12/20 (60%) and 15/19 (79%) patients respectively. Nine of them showed a complete and durable regression of the lesions at 12 months and at 24 months. The strength of the vaccine-induced HPV16-specific T-cell response was significantly higher in the group of patients with a complete regression of their lesions compared to non-responders. Patients with large lesions were less likely to experience a complete clinical response than patients with small lesions and we ascribe this to a larger proportion of vaccine induced HPV-specific regulatory cells in the patients with large lesions.
In conclusion, treatment with the HPV16 SLP vaccine is clearly effective in patients with established VIN disease. The SLP platform lends itself for development of therapeutic vaccines against many other chronic infections and non-viral cancers. In patients with cancer, it is attractive to combine this type of vaccination with immunogenic forms of cancer chemotherapy and with immuno-modulatory drugs.

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Profiling of metastatic melanoma to identify strategies to overcome immune resistance in the tumor microenvironment

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Immunotherapeutic approaches for the treatment of melanoma, such as tumor antigen-based vaccines, can frequently boost immune responses as measured in the peripheral blood. However, tumor responses are typically seen in only a minority of patients. This observation has prompted careful analysis of the tumor microenvironment to probe for biologic correlates to clinical response and also to identify mechanisms of tumor response versus resistance. Patients with advanced melanoma treated on two different vaccine platforms had pre-treatment tumor biopsies analyzed by Affymetrix gene expression profiling. Supervised hierarchical clustering was performed based on favorable or unfavorable clinical outcome. An expanded cohort of tumors was analyzed to increase the sample size and better understand patterns that segregate into subtypes. Two major categories of melanoma metastases have been identified. One subgroup of patients has an inflamed phenotype that includes expression of chemokines, T cell markers, and other immunoregulatory molecules. Clinical responders to melanoma vaccines appear to fall within this subset. This group also contains the highest expression of negative regulatory factors, including PD-L1, IDO, and FoxP3, suggesting that these immune suppressive mechanisms may dominantly inhibit anti-tumor T cell function in those patients. In addition, absence of B7 expression suggests that classical T cell anergy also may be operational. Preclinical experiments have confirmed a critical role for all 4 of these mechanisms in limiting anti-tumor T cell efficacy in vivo, giving candidate treatment strategies for translation back into the clinic. These include IDO inhibitors, anti-PD-1 mAbn, and approaches to deplete $CD25^+$ Tregs. A second subset of patients is represented by tumors which are non-inflamed and lack chemokines for T cell recruitment. Therefore, a major barrier in these cases appears to be failed T cell migration into tumor sites. Experimental strategies to augment T cell migration can have profound anti-tumor effects in preclinical models. Finally, presence or absence of inflammation was associated with a type I IFN transcriptional signature, and murine experimental models have confirmed a critical role for type I IFN signaling in mediating innate immune sensing of a growing tumor in vivo. Our results confirm that molecular profiling of melanoma metastases may be useful as a predictive biomarker for response to melanoma vaccines. The patterns of gene expression observed in distinct subsets of patients suggest specific strategies to overcome resistance to immune destruction at the level of the tumor microenvironment.

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Integrating immunology with cancer biology and medicine

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To have an organized and understandable picture of the meaningful interactions between two highly complex systems such as the immune system and the developing tumor, we applied systems biology to reveal an integrative cancer immunology network that will underline interactions between high numbers of elements and provide testable functional hypotheses (1). This was performed on large cohorts of primary tumors of human colorectal cancer patients of all stages in which not only the immune cell populations were quantified, but also their location in the center and the invasive margin was assessed as well as their functional orientation measured by cytokine, receptor or marker gene expression. This huge set of data was crossed with the expression of non-immune factors, such as vascularization components in the tumor and correlated with clinical outcome. Meaningful markers were then used as a matrix to predict the other components associated with good or bad prognosis and to generate testable hypotheses to understand the shaping of an efficient immune reaction.

A huge set of data analyzed in a non-biased fashion on a library of 959 colorectal tumors evidenced that high densities of memory T cells were associated with good prognosis for disease-free and overall survival. A careful examination of the immune infiltrate revealed that topological and functional coordination was associated with clinical outcome. A coordinated expression of genes involved in Th1 and cytotoxic orientation of infiltrating lymphocytes was associated with high density of memory cells and good prognosis (2,3). Once defined what is the "in situ" immune pattern in terms of location, functionality and maturation, i.e. the immune contexture, it can be analyzed how it interacts with other components of the tumor microenvironment. Vascularization is a well-known element involved in tumor progression and VEGF is a target for therapy, particularly in colorectal cancer. Expression of VEGF gene which was not found to be, by itself, a prognostic factor profoundly influenced clinical outcome in regards to the immune contexture. Indeed, high VEGF expression in the context of a coordinated Th1/cytotoxic T cell response counteracted the positive impact of the latter, patients with high Th1/cytotoxic T cell infiltrate and low VEGF being of good prognosis but those with high VEGF gene expression behaving the patients with non-coordinated T cell infiltrates (4).

An obvious question that has both basic and potential therapeutic incidence is what shapes an efficient immune reaction. It was again approached by systems biology based on a novel gene oncology based tool called Clue GO

that allowed to establish a gene-gene correlation network based on experimental data integrated with "in situ" informations (1). The top 65 genes predicted to interact with the ones that were identified as correlating with clinical outcome segregated into several major categories such as T cell activation and differentiation, B cell activation, negative regulation of immune responses, innate and inflammation responses, migration or were tumor cell related. In fact, the highest prediction score concerned the chemokine genes CX3CL1, CXCL9 and CXCL10. Indeed, when experimentally tested, high expression of these genes in the tumors correlated with high densities of Th1/cytotoxic memory T cells and with favorable prognosis. An adaptive immune response is characterized by antigen recognition and repertoire selection. However, numerous studies failed to identify a restricted T cell repertoire in human tumors. Indeed, applying an immunoscope analysis on T cells extracted from colorectal tumors, a highly disease polyclonal repertoire was found. Applying a hierarchical clustering of correlation matrix of the VB families and CDR length of T cells, purified from resected tumors revealed that a restricted repertoire clustered with the expression of CX3CL1, CXCL9 and CXCL10.

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Dormancy in normal and malignant stem cells

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Adult stem are required to maintain highly regenerative tissues such as the skin, the intestinal epithelium and the hematopoietic system. Mouse hematopoietic stem cells (HSCs) are the most well characterized somatic stem cell to date, and serve as a model for understanding other adult stem cells present in the mammalian body. Using two types of label-retaining assays we have identified a long-term dormant population within the most immature HSCs (Lin-Sca1+cKit+CD150+CD48-CD34-). Computational modeling suggests that dormant HSCs (d-HSCs) divide about every145 days, or 5 times per lifetime. d-HSCs harbor the vast majority of multi-lineage long-term self-renewal activity. While they form a silent reservoir of the most potent HSCs during homeostasis, they are efficiently activated to self-renew in response to bone marrow injury or G-CSF stimulation. After re-establishment of homeostasis activated HSCs return to dormancy, suggesting that HSCs are not stochastically entering the cell cycle, but reversibly switch from dormancy to selfrenewal under conditions of hematopoietic stress (1,2).
One of the reasons cancer stem cells are thought to escape anti-proliferative chemotherapy is their relative dormancy (3). We now have shown that treatment of mice with Interferon-alpha family leads to the activation and proliferation of dormant HSCs in vivo, which sensitizes them to chemotherapy drugs. HSCs lacking either the interferon-a/b receptor, STAT1 or Sca-1 are insensitive to IFNa stimulation, demonstrating that STAT1 and Sca-1 mediate IFNα induced HSC proliferation (4). The implications of these results for the design of strategies to target dormant CML stem cells not targetable by imatinib alone will be discussed.

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Epithelial stem cells during DNA damage and cancer initiation

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Adult stem cells (SCs) are at high risk of accumulating deleterious mutations because they reside and self-renew in adult tissues for extended periods of time. Little is known about how adult SCs sense and respond to DNA damage within their natural niche. Using mouse epidermis as a model (1), we define the functional consequences and the molecular mechanisms by which adult SCs respond to DNA damage. We found that multipotent hair follicle bulge SCs exhibit increased resistance to DNA damage-induced cell death by the higher expression of the anti-apoptotic gene Bcl2 and the transient p53 stabilization following DNA damage in bulge SC. The attenuated p53 activation is the consequence of a faster DNA repair activity mediated by a higher non-homologous end joining (NHEJ) activity. Since NHEJ is an error prone mechanism, this novel characteristic of adult SCs may have important implications in cancer development (2).

For the vast majority of cancers, the cell at the origin of tumour initiation is still unknown. We are using mouse genetics to identify cells at the origin of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), the two most frequent skin cancers in human. Using mice conditionally expressing constitutively active Smoothened mutant (SmoM2), we activated hedgehog signalling in different cellular compartments of the skin epidermis, and determined in which epidermal compartments hedgehog activation induces BCC formation. Activation of SmoM2 in hair follicle bulge SC and their transient amplifying progenies did not induce cancer formation, demonstrating that BCC do not originate from bulge SC as previously thought. Using clonal analysis, we found that BCC arise from long-term resident progenitor cells of the interfollicular epidermis and the upper infundibulum. Our studies uncover the cells at the origin of BCC in mice and demonstrate that expression of differentiation markers in tumour cells is not necessary predictive of the cancer initiating cells (3,4). Using mice conditionally expressing constitutively active Ras mutant, we activated Ras pathway in different cellular compartments of the skin epidermis, and determined from which epidermal compartments SCC arises. Our findings demonstrate these two skin cancers have different cellular origin. The relevance of our studies for the diagnosis, prognosis and treatment of human cancer will be discussed.

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Leukemia initiating cells are frequent and oligoclonal in de novo resistant acute lymphoblastic leukemia

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Whether acute lymphoblastic leukemia (ALL), the most frequent malignancy of childhood, arises and is maintained from a limited compartment of leukemia initiating cells (LIC) remains a highly controversial and an important question. The LIC compartment may constitute a reservoir for selection of resistant clones to occur under selective pressure by chemotherapy. Data obtained by intravenous transplantation of sorted ALL subpopulations in immunocompromised NOD/SCID mice suggested LIC enrichment for high risk ALL in a restricted immature stem cell compartment, characterized by a CD34+ and CD19- phenotype (1). However, this concept has been challenged by observations in syngeneic mouse models consistent with a LIC frequency of 20 percent (2). Moreover, orthotopic intrafemoral injection of sorted leukemia cells from immature ALL compartments (CD19-, CD34+) or from mature compartments (CD19+, CD34+) consistently repopulated leukemia in NOD/SCID mice (3), suggesting that most cells in leukemia samples may have leukemia initiating capacity. The characterization of the hematopoietic hierarchy in ALL and the consequences of selective pressure by chemotherapy on the clonal composition of ALL populations have important implications for preclinical disease modeling.

We have established a leukemia xenograft model in immunodeficient NOD/scid*IL2R*γ_{null} (NSG) mice that served to identify and validate a new therapeutic agent for chemosensitization of de novo resistant ALL (4), using samples that were derived from a group of patients with very high-risk precursor B-cell ALL based on persistence of minimal residual disease (VHR-ALL by MRD) on a uniform international treatment protocol. This model is well suited to address the aforementioned questions. Engraftment kinetics (median time to leukemia of 8 weeks for VHR-ALL) were unchanged with concordant immunophenotypes for 95% of the leukemia-associated cell surface lineage markers after up to five passages in NSG mice. Serial dilution transplantation assays showed that 100 unsorted ALL cells were sufficient to generate leukemia in 4/6 cases with VHR-ALL, suggesting a high frequency of leukemia initiating cells in high risk ALL. To evaluate clonal stability in xenografted ALL cells we used single nucleotide polymorphism microarrays and identified up to five focal copy number aberrations (CNAs) per case, mostly recurrent lesions in ALL. Most CNAs were maintained in primografts, with the exception of two alterations corresponding to minor subpopulations. In all samples, 0-2 additional CNAs were detected immediately after the first passage in mice, suggesting the possibility of clonal selection. Following individual cells with fluorescent in situ hybridization (FISH) to detect CNAs in a region with frequent mono-or biallelic aberrations in high risk ALL (chromosome 9p21), we confirmed that if clonal selection occurs in some cases, the subclone is detectable in the original sample. The oligoclonal composition of primograft passages remains remarkably stable throughout all samples. I will discuss these results in the context of relevant data in the field and illustrate how we are using this platform for preclinical testing of new therapeutic agents and biomarkers as part of a large international clinical trial for the treatment of relapsed childhood ALL.

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Intrinsic Therapeutic Resistance of Breast Cancer Stem Cells

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Despite the recent advances in breast cancer treatment, many patients relapse after an initially favorable response to chemotherapy and radiation therapy. There are at least two possible, not necessarily mutually exclusive, explanations for this observation. One is that all cancer cells can acquire resistance resulting in decreased overall sensitivity to therapy over time. Alternatively, a subpopulation of cells with tumorigenic potential is intrinsically resistant to therapy. To distinguish between the two options, we have used a unique $p53^{null}$ murine breast cancer model that allowed us to identify a tumor initiating subpopulation of cells (1). By comparing the transcriptome of this subpopulation with that of the bulk tumor cells, we found that the tumor initiating cells(TICs) expressed higher levels of DNA–damage response genes and DNA repair genes. Consistent with these results are the *in vitro* and *in vivo* demonstration of more efficient DNA damage repair in TICs than in the bulk tumor. In addition, we have been able to selectively sensitize TICs to radiation treatment *in vivo* using both thermal enhancement with optically activated gold nanoshells (2), as well as an Akt inhibitor, which we demonstrated directly inhibits the canonical Wnt/ß-catenin pathway in TICs (3). These findings support the hypothesis that tumor–initiating "cancer stem cells" may be intrinsically more resistant to DNA damage explaining in part tumor resistance to radiation therapy and chemotherapy. Further support for this hypothesis comes from our study of paired human breast cancer core biopsies before and after chemotherapy; this demonstrated that tumorigenic cells were intrinsically chemoresistant (3). Such cells are characterized by a gene signature otherwise found mainly in human breast tumors of the recently identified $claudin^{low}$ and metaplastic subtype; this subtype expresses many mesenchyme–associated genes (4). Surprisingly, post–treatment residual tumors contained a higher fraction of $claudin^{low}$ cells, consistent with therapy–mediated enrichment of resistant cells. Such cells expressed higher levels of markers characteristic of epithelial–to–mesenchymal transition (EMT), and in claudinlow tumors developed in the $p53^{null}$ murine breast cancer model, there was a concomitant loss of specific miRNAs known to regulate EMT. Thus, a small subset of cells expressing mesenchymal markers appears to be responsible in part for the intrinsic resistance to therapy.

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Poster Abstracts

1

A novel long patch base excision repair of 8-oxo-guanine coordinated by MutY glycosylase homologue (MUTYH) and DNA polymerase λ

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The potentially mutagenic A:8-oxo-G mispair synthesized by the replicative DNA polymerases (pols) δ and ε escapes proofreading. The mismatch is recognized by the MUTYH that removes the A, leaving the lesion on the template strand. The subsequent repair mechanism allowing the formation of C:8-oxo-G base pair remains to be clarified. We have recently shown that the base excision repair enzyme DNA pol λ, together with the auxiliary proteins replication protein A (RP-A) and proliferating cell nuclear antigen (PCNA), has the unique ability among human DNA pols to efficiently incorporate a C opposite an 8-oxo-G, with error frequencies in the range of 10-3 (1, 2). In this work we identified the critical cellular components that specifically bind to DNA containing A:8-oxo-G mispairs and subsequently reconstituted a novel error-free pathway of 8-oxo-G. We showed specific binding of MUTYH, DNA pol λ, PCNA, FEN1 and DNA ligases I and III from human whole cell extracts to A:8-oxo-G DNA, but not to undamaged DNA. Based on this observation, we fully reconstituted a pathway for the repair of A:8-oxo-G mispairs. In a MUTYH and apurinic endonuclease 1 initiated reaction, DNA pol λ in the presence of RP-A and PCNA incorporated dCTP opposite 8-oxo-G and added one nucleotide. The repair pathway was completed by FEN1 and DNA ligase I. These results identify a novel pathway, where a replication A:8-oxo-G mispair product is correctly repaired via MUTYH/DNA pol λ dependent long patch base excision repair (3). DNA pol λ has the unique property to act at a damaged DNA (8-oxo-G) for faithful repair.

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2

Human melanoma contains CD271-positive melanoma stem cells associated with metastatic potential

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Human melanoma is composed of distinct cell types reminiscent of neural crest derivatives and contains multipotent cells that express the neural crest stem cell markers CD271(p75NTR) and Sox10. Upon transplantation into Nude or NOD/SCID mice, CD271-positive but not CD271-negative cells formed tumors that fully mirrored the heterogeneity of the parental melanoma and could be passaged more than 5 times. In contrast, in more immunocompromized NOD/SCID/IL2rγnull (NSG) mice, or in NK cell-depleted Nude or NOD/SCID mice, both CD271-positive and CD271-negative tumor cell fractions established tumors. However, tumors resulting from either fraction did not phenocopy the parental tumors, and tumors derived from the CD271-negative cell fraction could not be passaged multiple times. This observation argues against the use of NSG mice in the context of human cancer stem cell research. Together, our findings identify CD271-positive cells as melanoma stem cells. Our observation that a relatively high frequency of CD271/Sox10-positive cells correlates with higher metastatic potential and worse prognosis further supports that CD271-positive cells within human melanoma represent genuine cancer stem cells.

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3

Disruption of a negative feedback loop promotes growth factor independence and chemotherapy resistance in Ovarian Cancer

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Evasion of apoptosis has been described as one of the hallmarks of cancer and is often mediated by mutations in key cellular genes. This allows the cancer cell to survive metabolic stress conditions such as limited availability of growth factors, nutrients and oxygen. Another consequence of apoptosis evasion is an increased resistance against chemotherapy treatment. Recently, the prefoldin URI has been shown to be an important component of a mitochondria based negative feedback loop which controls the cellular apoptotic threshold. Under conditions of hyperactive S6K1 survival signaling, protein complexes between the pro-apoptotic phosphatase and its natural inhibitor URI are disrupted via phosphorlyation of URI at Ser-371. Released PP1γ becomes active and can in turn dephosphorylate S6K1 and BAD, thereby lowering the threshold for apoptosis. Based on that model we addressed the question if this negative feedback loop is disrupted in cancer cells as a mean to promote apoptosis evasion.

Here we identify URI as an oncogene amplified and overexpressed in ovarian cancer cell lines and human ovarian carcinomas. The amplification and overexpression of URI characterizes a subset of carcinomas with aggressive behaviour and correlates with poor disease-specific patient's survival and resistance to platinum-based chemotherapy. URI is the only 'addicting' oncogene at chromosome 19q12 selectively required for the survival of ovarian cancer cells with increased URI copy-number as only these cells undergo a strong apoptotic response and decreased tumor growth upon URI depletion. By constitutively detaining PP1γ in inactive complexes the upregulation of URI leads to a maintained S6K1 survival signalling under growth factor limiting conditions. This enhanced signalling output is responsible for increased cell survival and enhanced tumour cell growth observed in colony formation assays and mouse xenograft models. As a direct consequence of disrupted negative feedback signalling URI-amplified cancer cells become partially resistant to pharmacological inhibition of the up-stream kinase mTOR by rapamycin. Moreover, sustained S6K1 survival signalling under cis-platin treatment mediates resistance of URI amplified and URI overexpressing cancer cells to cisplatin and explains at least in part why patients with URI amplified carcinomas frequently fail to respond to platinum-based chemotherapy.

Thus, oncogenic activation of URI defines a novel molecular mechanism for promoting cell survival through disabling negative feedback inhibition. These findings imply that the URI/PP1γ complex might be an interesting target for mechanism-based therapies in patients whose tumors are characterized by URI amplification.

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4

Muscle Precursor Cells inhibit tumor growth upon paracrine secretion of TNF alpha

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Introduction. Autologous cells have the potential to provide replacement organs made from patients own cells. Muscle precursor cells (MPC), are skeletal muscle cells sources capable of regenerating muscle fibers, and therefore investigated for the treatment of several muscular diseases. A putative problem is that patients in need of engineered organs are generally older and therefore at risk of cancers. Yet, the safety of the treatment with such compelling multipotent cells and its interactions with cancer has not yet been investigated. In this study, we evaluate in vitro the impact of MPC over the growth and malignant potential of different prostate carcinoma and sarcoma cell lines.
Materials and Methods. In order to assess these bilateral interactions we used a cell co-culture system in vitro and determined growth rate, tumor apoptosis and cell cycle arrest, by FACS, immunocytoschemistry (ICC) and mRNA expression levels. Muscle differentiation was tracked by fiber formation assay, ICC, Western blot and RTPCR.
Results. Differentiating muscle in the proximity of tumor caused a significant cancer growth rate decrease, induced cell cycle arrest and apoptosis (p21 and Caspase3 up-regulation) by secretion of TNF alpha and reexpression of BIN1, consequently by blocking of tumor c-Myc activity. Conversely, muscle progenitor cell differentiation was significantly increased in vitro by the presence of tumor cells, inducing formation of well organized myotubes with all the features of normal and functional muscle. This tumor suppressed could be blocked in large part by TNFα inhibition.
Discussion and Conclusions. These results indicate that MPCs, while differentiating, do secrete TNF alpha and thereby inhibit tumor growth, inducting apoptosis and tumor cell death. These results underline the safe use of MPC for treatment of Urinary Incontinence in patients with previous cancer and/or in tumor proximity.

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5

Engineering melanoma progression in a humanized environment 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 immuno-compromized rats consistently resulted in 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 explored in a humanized, clinically relevant context.

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6

Mouse models of inducible cancer to study the immunosuppressive tumour environment

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Background: Despite the fact that spontaneous tumour-specific immunity can be detected in tumour-bearing mammals or that such immunity can be induced or boosted by immunisation, immune surveillance fails in most occasions to control or eliminate the tumour. It is becoming increasingly clear that the tumour environment delivers suppressive signals to the immune system, which impair the effector function of tumour-infiltrating lymphocytes. The identity of these signals, their targets and their mode of action, however, are largely unresolved.
Aim: Using a mouse model of inducible cancer in a tissue specific fashion, we aim to identify mechanisms that suppress the action of tumour-specific T cells. We will analyse mechanisms involved in central and peripheral tolerance induction as well as local factors within the tumour that deviate immune surveillance.
Experimental design: We will establish an inducible Cre/LoxP-based model system to express the immunologically well-characterized oncogene SV40 large T antigen plus luciferase in a tissue specific fashion. We will induce expression in pancreatic acinar cells, melanocytes, B cells or in oligodendrocytes, which will result in tumours of the pancreas, skin, B cells or brain, respectively. Simultaneous expression of luciferase and SV40T allows us to detect small developing tumours at an early stage. First, we will assess quantitative and qualitative aspects of the T cell response during tumour development in the draining and non-draining lymph nodes, the spleen and in the tumour. Based on previous publications, we expect impaired effector function within the tumour, but probably also peripherally, with increasing tumour burden. Second, we will investigate the contribution of regulatory T cells and of suppressive molecules such as IL-10, TGF-beta, CTLA-4, VEGF or PD-1. The immunological analysis of these manipulated mice will concentrate on the phenotype and function of dendritic cells and of T cells in the tumour and at peripheral sites. These data will be combined with data on tumour growth. Third, after having identified factors involved in defective immune surveillance, we will test therapeutic strategies to overcome or bypass tumour-specific unresponsiveness. In addition, by comparing four fundamentally different target tissues, we may find mechanisms that are active in every malignancy and some that are organ-specific.

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7

Gene expression profile of stroma reactive to prostate cancer osteoblastic bone metastasis identifies cancer progression

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Cancer cells modulate their adjacent stroma to build a supportive microenvironment for primary and metastatic cancer progression. Several gene expression studies have been performed to characterize this tumor reactive stroma in order to identify molecular determinants of cancer progression. However, the relative contribution of the stromal compartment to the gene expression cannot be readily assessed by gene expression profiling of bulk tumour tissue. Consequently, the molecular determinants of the stromal support, especially in bone metastasis, have remained largely elusive.
In this study, we used tissue compartment-specific transcriptional profiling (TCTP) to obtain a bone metastasis-associated stroma transcriptome (BMST) in mouse xenograft models of human prostate cancer (PCa) osteoblastic bone metastasis. This novel method exploits the divergence between human and mouse transcriptomes in combination with a highly mouse-specific gene array platform, and the exclusion of cross-hybridizing cDNA probes by a computational mask. The mouse, stroma-specific gene expression profile

derived from bones xenografted either with C4-2B4 or with VCaP cells was compared to that derived from sham-operated bones. A minimal percent (4.3%) of the total mouse genes represented on the mouse cDNA array was excluded from analysis because cross-hybridizing with human RNA. A total of 655 and 582 genes were differentially expressed (≥ 2-fold change) at high stringency in C4-2B (FDR = 1 x 10 –5) and VCaP (FDR = 3 x 10 –5) reactive stroma, respectively. C4-2B- and VCaP-xenografted bones shared 323 common genes, which represent a BMST. Among this BMST, 206 (63.8%) genes were up- and 117 genes (36.2%) were down-regulated. Gene ontology clusters of up-regulated genes related them to extracellular matrix, cell-matrix interactions, angiogenesis and various signalling pathways. The down-regulated genes were involved in haematopoiesis, mitosis and DNA repair. The stromal up-regulation in the tumour-bearing bones of a list of selected genes was validated by quantitative RT-PCR using mouse-specific primers. Immunohistochemistry confirmed stromal expression of the proteins encoded by some genes in this list both in cancer cell xenografted bone, and in human primary and bone metastatic PCa and mammary carcinoma. Co-cultures of primary mouse osteoblasts with C4-2B4 cells showed that induction of the selected genes in osteoblasts is dependent on direct contact with cancer cells. Meta-analysis of previous gene expression studies revealed that a BMST cluster marks the transition from localized to invasive carcinoma in multiple cancer types and may negatively correlate with patient survival.
TCTP reliably provided an osteoblastic BMST, which is reminiscent of the desmoplastic response seen in wound healing and cancer. A gene cluster within the BMST may predict cancer progression and patient survival. Additionally, the proteins encoded by some genes within this cluster are potential biomarkers of cancer progression.

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8

The immunomodulatory role of endogenous glucocorticoids in ovarian cancer

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The enzyme 11 beta-hydroxysteroid dehydrogenase I (11bHSD1) is essential for the conversion of biologically inactive cortisone into active cortisol. Using immunohistochemistry and real-time PCR, we found this enzyme to be highly expressed in human and murine ovarian cancer tissue. Immunofluorescent double staining revealed a co-localization of 11bHSD1 with CD14, CD68, and CD85, but not with EpCAM. Expression of 11bHSD1 can thus be attributed to tumour associated macrophages (TAM) or myeloid derived suppressor cells (MDSC). In line with increased local activation of endogenous glucocorticoids, luminescent immunoassays confirmed elevated cortisol levels in serum from ovarian cancer patients as compared to healthy controls. High amounts of endogenous cortisol could further be detected in ascites and tissue exsudates from ovarian cancer patients. Considering that cortisol has strong suppressive effects on all kinds of immune cells, we hypothesize that the activation of endogenous glucocorticoids by TAM or MDSC may contribute to a highly immunosuppressive microenvironment and thereby to the immune escape of ovarian cancer. This hypothesis is now being tested in PTENloxP/loxP; loxP-Stop-loxP-krasG12D mice which spontaneously develop ovarian cancer after intra-bursal injection of adenoviral Cre recombinase. The ongoing and projected experiments involve adoptive transfer of glucocorticoid receptor knock out immune cells as well as pharmacological inhibition of 11bHSD1 which shall be combined with various immune stimuli. In a first functional in vivo assay, the adoptive transfer of glucocorticoid receptor-deficient T cells led to increased immune cell infiltration of the tumour tissue - which did not translate into prolonged survival. Instead, infiltrating T cells assumed mostly a Foxp3+ (regulatory) phenotype and survival was even shortened. We thus propose that endogenous glucocorticoids exert immunomodulatory functions in ovarian cancer. Their putative role in tumour immune escape, however, needs to be assessed in context of further tolerogenic mechanisms that may be simultaneously present.

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9

Comparison of phenotypic properties between side population derived- and recurrent tumors in malignant pleural mesothelioma

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Asbestos related malignant pleural mesothelioma (MPM) is a devastating disease with increasing incidences and poor response to current treatments. Inhaled asbestos fibres accumulating in the pleural space lead to chronic tissue repair entailing oncogenic events essential for tumor development. We made the hypothesis that the oncogenic damage may occur in cells with mesothelium precursor phenotype, recruited to repair tissue injury and that these cells would be associated with chemoresistance and tumor recurrence as observed in other cancer types.
Using the side population (SP) functional assay we identified SP cells with self-renewal properties and increased chemoresistance in MPM cell lines and tumor derived primary cell cultures. Compared to the non SP fraction, the SP fraction led to the development of tumors shifting to a mesothelium precursor phenotype characterised by mesenchymal morphology, being Wilms tumor 1 (WT1) negative and podoplanin positive, and having a tendency of increased tumorigenicity. The same phenotypic shift was observed in patients at the time of tumor relapse after chemotherapy. Furthermore the SP cells were enriched in CD105 (endoglin) -/low expressing cells. The latter were small sized and had increased tumorigenicity compared to CD105 high cells. Taken together our results support the hypothesis that MPM CD105-/low, chemoresistant, small sized SP cells could be responsible for tumor recurrence in patients. Further characterization of mechanisms of chemoresistance and self-renewal in these cells should lead to new targets for MPM treatment.

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Radiotherapy promotes tumor-specific effector CD8+ T cells via DC activation

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Radiotherapy is an important treatment for cancer. The main mode of action is thought to be the irreversible damage to tumor cell DNA, but there is evidence that irradiation mobilizes tumor-specific immunity and recent studies showed that the efficacy of ablative radiotherapy depends on the presence of CD8+ T cells. We show here that the efficacy of ablative radiotherapy crucially depends on dendritic cells and CD8+ T cells, whereas CD4+ T cells or macrophages are dispensable. We show that local ablative irradiation results in activation of tumor-associated dendritic cells that in turn support the development of tumor-specific effector CD8+ T cells, thus identifying the mechanism that underlies radiotherapy-induced mobilization of tumor-specific immunity. We propose that – in the absence of irradiation – the activation status of dendritic cells rather than the amount of tumor-derived antigen is the bottleneck, which precludes efficient anti-tumor immunity.

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Identification of New Cellular Targets for Lung Cancer Therapy

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From in vitro studies it is known that inhibition of REV3, which is the catalytic subunit of the translesion polymerase ζ (zeta), reduces formation of resistance and increases sensitivity of human cells to cisplatin. Recent findings of our laboratory revealed that inhibition of REV3 expression suppresses growth of mesothelioma, lung- and breast cancer cells whereas normal cells are not affected. The purpose of this project is to identify genes whose inhibition will not only reduce but completely abolish cancer cell growth in a REV3-deficient background whereas normal cells would not be affected.
Today the concept of chemotherapy is based on the assumption that a high load of DNA damage induced by the maximal tolerated dose of chemotherapy will kill preferentially cancer cells. This crude approach is only partially successful because of the problem of resistance development induced by the DNA damage. Therefore we want to develop a method for killing tumor cells without inducing additional DNA damage. This is based on a phenomenon called "the principle of synthetic lethality", which is that two pathway deficiencies alone can be tolerated but become lethal when combined. Cancer cells are already deficient for cell cycle checkpoint control, and additional inhibition of DNA repair will render cancer cells extremely sensitive to additional stress by yet to be identified molecular pathways. To find such pathways we will perform sets of global screens to identify targets affecting viability of REV3-deficient cancer cell. These screens will be done with the Dharmacon siARRAY
Human Genome Library. This library includes siRNA reagents that target over 21,000 unique genes in the human genome.
The specific aims are:
1. Generation of clones of a non-small cell lung cancer cell line A549 with reduced REV3 expression.
2. In collaboration with the group of Dr. Victor van Beusechem and Prof. Egbert Smit at the Vrije University Medical Center Amsterdam we will design and run whole genome siRNA screens to identify targets affecting specifically the viability of REV3-deficient cancer cells.
3. In vitro evaluation and confirmation of the screening results.

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Structural insights into oncogene-induced DNA replication stress

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The DNA damage response is a critical anti-tumour barrier and prevents the proliferation of cells with potentially hazardous genetic alterations. It acts early in tumourigenesis, as its activation was observed already in pre-cancerous lesions of various organs. The activation of the DNA damage checkpoint in these lesions was ascribed to oncogene-induced deregulation of DNA synthesis, or "replication stress". Although the indirect consequences of replication stress, i.e. cell cycle arrest and oncogene-induced senescence, have been elucidated to some extent, our understanding of the underlying molecular events is extremely vague. This is mainly due to the lack of information on the in vivo DNA structures that are generated under such conditions.
The replication stress phenotype can be reproduced in cell culture by overexpression of various oncogenes influencing DNA replication, e.g. Cyclin E, Cdc25A. We are exploiting these systems to identify oncogene-associated defects in DNA replication. Flow cytometric measurements indicate a substantial effect of oncogene deregulation on bulk DNA synthesis. Preliminary DNA fibre labelling experiments suggest a marked impact of oncogene expression on the progression of individual replication forks early after oncogene induction, coinciding with activation of the CHK1 kinase. Pulse field gel electrophoresis shows increasing DNA breakage upon oncogene overexpression at later timepoints, which is associated with phosphorylation of CHK2. For Cdc25A, we find that these double strand breaks are, at least partially, due to enzymatic processing during DNA replication. Electron microscopic analysis of in vivo replication intermediates isolated from our model systems is underway.

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The traditional Chinese medicine derived compound ascaridol shows strong differential cytotoxicity in nucleotide excision repair-deficient cells

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Targeting synthetic lethality in DNA repair pathways has become a promising anti-cancer strategy. The most well described example is the synthetic lethal interaction of poly(ADP-ribose) polymerase inhibitors within cancer cells showing defects in the DNA double strand break repair pathway by mutations in BRCA1/2. There is however little knowledge on such lethal interactions in nucleotide excision repair (NER)-deficient cells although many cancer types are known to harbor mutations in NER genes. We therefore decided to search for novel synthetic lethal interactions in NER-deficient cells. As a first step, we used two XPC- and ERCC6-deficient cell lines to screen traditional Chinese medicine (TCM) drugs for differential cytotoxic effects in repair-deficient versus repair proficient cells. The cytotoxicity screening of 72 drugs revealed 13 compounds that killed NER-deficient cells more efficiently than proficient cells. Five of these TCM drugs were further analysed for IC50 values, effects on cell cycle distribution, and induction of DNA damage. We identified the monoterpene peroxide ascaridol as the most effective compound with a more than 1000fold higher cytotoxicity in NER-deficient cells compared to normal cells (IC50 for NER-deficient cells = 0.03 µg/ml, for repair proficient cells >30 µg/ml). Furthermore, NER-deficiency combined with ascaridol treatment lead to an increased percentage of apoptotic cells as detected by flow cytometry and a 3fold higher DNA damaging activity as measured by the alkaline comet assay. We confirmed these results in a second set of NER-deficient and -proficient cell lines with isogenic background. Finally, ascaridol was characterized for its ability to generate oxidative DNA damage. The drug lead to a dose-dependent increase in intracellular levels of reactive oxygen species and only NER-deficient cells showed a strongly induced amount of oxidized bases determined as formamidopyrimidine DNA glycosylase-sensitive sites by the comet assay. In summary, ascaridol is a cytotoxic and DNA damaging compound which generates intracellular reactive oxidative intermediates and which selectively affects NER-deficient cells. This could provide a new therapeutic option to treat cancer cells with known mutations in NER genes.

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Inhibition of REV3 expression induces persistent DNA damage and growth arrest in cancer cells

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Rev3 is the catalytic subunit of DNA translesion synthesis polymerase ζ. Inhibition of Rev3 increases the sensitivity of human cells to a variety of DNA damaging agents and reduces the formation of resistant cells. Surprisingly, we found that silencing of REV3 per se suppresses colony formation of lung (A549, Calu-3), breast (MCF-7, MDA-MB231), mesothelioma (IL45 and ZL55) and colon (HCT116 +/-p53) tumor cell lines whereas normal cell lines (AD293, LP9-hTERT) and the normal mesothelial primary culture (SDM104) are less affected. Inhibition of REV3 expression in cancer cells leads to an accumulation of persistent DNA damage as indicated by an increase in phospho-ATM-, 53BP1- and phospho-H2AX-foci formation, subsequently leading to the activation of the ATM-dependent DNA damage response cascade. REV3 inhibition in p53-proficient cancer cell lines results in a G1-arrest and induction of senescence as indicated by the accumulation of p21 and an increase in senescence-associated (SA)-β-Galactosidase activity. In contrast, REV3 inhibition in p53-deficient cells results in a G2/M-arrest and in an elevated frequency of aneuploid cells.
Our findings reveal that REV3 inhibition per se suppresses cancer cell growth whereas normal cells are less affected, thus identifying REV3 as a potential target for a cancer-specific therapy.

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Involvement of the CXCL12/CXCR4/CXCR7 axis in the malignant progression of human neuroblastoma (NB)

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NB is a devastating childhood neoplasm for which no efficient treatment is available for late stage tumours. Chemokines and their receptors, in particular the CXCR4/CXCL12 axis, have been implicated in tumour progression. We previously reported a tumour type-specific and microenvironment-related growth-promoting role for the CXCR4 receptor. Such growth-promoting effects were highly significant only when NB cells were orthotopically injected in the adrenal gland of nude mice. This finding highly suggested a pivotal cross-talk between the CXCR4-expressing aggressive tumour cells and the associated microenvironment. The recent description of CXCR7 as a second CXCL12 receptor, add to the CXCL12/CXCR4 chemokine/receptor axis a new player, which function remains to be determined.
In search for specific microenvironment-related effects, which might cooperate with CXCR4-mediated NB tumour growth, we addressed the role and participation of CXCR7. Although reported to confer atypical properties to cancer cells, the role of CXCR7 in NB development and the cross-talk with the microenvironment is still unknown. A preliminary screening of a small panel of NB tissues of different stages and histology types for CXCR7 expression revealed a selective CXCR7 staining on the more differentiated cells of the tumour and on the associated adrenal tissue. In contrast, CXCR7 was only moderately expressed on NB cell lines, but was found to increase upon exposition of cells to differentiation agents.
From these preliminary observations, we propose that CXCR4 and CXCR7 may display two distinct and atypical roles in NB. Rather than a metastatic-promoting role identified in several other tumour systems, our data favour a tumour type-specific and growth-promoting influence for CXCR4, while CXCR7 may be implicated in NB maturation and interactions with the environment. These preliminary findings open new research perspectives for the role of the CXCL12/CXCR4/CXCR7 axis in the behaviour of NB that will be further explored in vitro and in vivo.

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DNA damage response (DDR) in stem cells: at the crossroad of ageing, cancer therapy and regenerative medicine

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Stem cells have the ability to self-renew and populate any given niche, differentiating into multiple cell types. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) hold great promise for the therapy of human diseases, but their functional similarity awaits further investigation. Small numbers of adult stem cells (ASCs) have been identified in differentiated tissues and their exhaustion has been linked to aging. A subpopulation of self-renewing pluripotent cells has also been identified in most tumors (cancer stem cells, CSCs).
The cellular pathways dealing with DNA damage and DNA replication stress have been recently shown to play a pivotal role in the maintenance of adult and cancer stem cells, and in restriction of reprogramming in iPSCs, but the underlying molecular mechanisms are largely unknown. The Lopes lab is specialized in molecular and structural studies on genome instability resulting from DNA replication stress, taking advantage of standard molecular and cell biology approaches and more specialized techniques to investigate DNA replication intermediates (RIs), such as DNA fibers/combing and in vivo electron microscopy.
In this project we plan to exploit this technological platform and precious collaborations within the University of Zurich to characterize the DNA damage response and DNA replication features of stem cells. We have started to analyze mouse ESCs, ASCs (hematopoietic stem cells, HSCs; neural crest stem cells, NCSCs), iPSCs and CSCs (melanoma and leukemia initiating cells) for the accumulation of endogenous DNA damage and for the basal activation of the DNA damage response. We will also test the recruitment of DNA repair factors, the modulation of cell cycle progression and the activation of central checkpoint kinases in response to exogenous stress. Finally, we will characterize their pattern of DNA replication (inter-origin distance, fork architecture and progression) and its modulation in response to DNA damage. Preliminary results suggest that ES cells in culture accumulate detectable markers of DNA damage (γH2AX), which are rapidly lost when these cells loose their stemness during differentiation. By our single molecule analysis of RIs, we aim to uncover possible alterations of the DNA replication program that may account for the DDR activation observed in these cells. We are also comparing different ESC culturing conditions and ESCs in early embryos (blastocysts) to assess whether DNA damage accumulation is intrinsic to these cells or if it results from in vitro culturing. We soon plan to extend these studies to cultured NCSCs and to dormant and active mouse HSCs, taking advantage of recently developed protocols to isolate activated HSCs upon injury. At a later stage we'll investigate cancer stem cells and genotoxic treatments on different stem cell populations (i.e. oncogene activation, chemotherapeutic drugs).
These studies could significantly advance our knowledge on the cellular mechanisms leading to stem cell exhaustion in aging individuals. Moreover, our results could shed light on oncogene-induced tumorigenesis within the stem cell compartment and on the resistance of cancer stem cells to tumor chemotherapeutics. Finally, we aim to contribute to the biological characterization of iPSCs, in order to elucidate their potential/limits for therapeutic approaches.

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MicroRNA deregulation in gastric MALT lymphoma

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Gastric marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) represents a distinct class of extranodal lymphoma that evolves against a background of chronic inflammation induced by persistent infection with the bacterium Helicobacter pylori. We have identified miR-203 as an important tumour suppressor miRNA in MALT lymphoma in connection with its known oncogene target ABL1. Pharmacological inhibition of ABL1 activity by imatinib blocked MALT lymphoma cell proliferation ex vivo and effectively eradicated tumours in vivo. Collectively, our observations suggest that ABL1 plays an important role in MALT lymphoma cell biology and support a novel potential application of imatinib in the treatment of MALT lymphoma. Low grade MALT lymphomas can eventually undergo high grade transformation to a more aggressive counterpart termed gastric diffuse large B-cell lymphoma (gDLBCL). At this stage the lymphomas

grow autonomously and are refractory to Helicobactereradication therapy. Genome-wide miRNA expression profiling revealed a characteristic set of Myc-repressed miRNAs to be specifically downregulated in human gDLBCL compared to MALT lymphoma and gastritis. Of the Myc-repressed miRNAs downregulated in malignant lymphoma, miR-34a showed the strongest anti-proliferative properties when overexpressed in DLBCL cell lines. We could further attribute the tumour suppressive effects of miR-34a to dysregulation of its target FOXP1. Transient knockdown of FOXP1 in DLBCL cell lines significantly impaired the proliferation of the tumour cells. Taken together, our findings elucidate a novel mechanism linking the aberrant expression of MYC and concomitant repression of miR-34a to FOXP1 deregulation in gDLBCL. We further propose miR-34a as a potential therapeutic target in the treatment of miR-34a-deficient, FOXP1-overexpressing lymphomas such as gDLBCL.

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Distinct caspase-10 isoforms play specific and opposing roles in the initiation of death receptor-mediated apoptosis

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Neuroblastoma (NB) is the second most common solid childhood tumour. The most aggressive NB cell lines are resistant to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). The cysteine protease caspase-8 is an essential executioner of the death receptor apoptotic pathway (TRAIL-R, FAS). The physiological function of its homologue caspase-10 remains poorly understood, and the ability of caspase-10 to substitute for caspase-8 in the death receptor apoptotic pathway is still controversial. Both caspases-8 and –10 are frequently down-regulated in paediatric tumours. The silencing of caspase-8 expression was shown to be responsible for the resistance of NB cells to TRAIL-induced apoptosis. We have previously demonstrated that stable restoration of caspase-8 expression fully restored TRAIL sensitivity in the caspase-8/-10 negative IGRN-91 NB cell line. Here we analysed the particular contribution of caspase-10 isoforms to death receptor-mediated apoptosis in neuroblastoma cells.

Silencing of caspase-8 in TRAIL-sensitive neuroblastoma cells resulted in complete resistance to TRAIL, which could be reverted by overexpression of caspase-10A or caspase-10D. Overexpression experiments in various caspase-8 expressing tumour cells also demonstrated that caspase-10A and caspase-10D isoforms strongly increased TRAIL and FasL sensitivity, whereas caspase-10B or caspase-10G had no effect or were weakly anti-apoptotic.
Further investigations revealed that the unique C-terminal end of caspase-10B was responsible for its degradation by the ubiquitin-proteasome pathway and for its lack of pro-apoptotic activity compared to caspase-10A and caspase-10D.
These data highlight in several tumour cell types, a differential pro- or anti-apoptotic role for the distinct caspase-10 isoforms in death receptor signalling, which may be relevant for fine tuning of apoptosis initiation.

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Identification of the Werner syndrome protein as a novel member of the error-free 8-oxo-guanine bypass pathway

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Reactive oxygen species (ROS) produced during normal cell metabolism readily attack DNA generating a variety of oxidized DNA bases with a high mutagenic potential. One of the most frequent oxidative DNA lesions is 7,8-dihydro-8-oxo-guanine (8-oxo-G) with a steady-state level of 4-10 lesions per 10^6 DNA bases in normal human tissue. All known replicative DNA polymerases (α, ε and δ) show a strong tendency to misincorporate an A

opposite an 8-oxo-G, leading to G:C to T:A transversion mutations. Interestingly, these transversion mutations are the most commonly found somatic mutations in lung, breast, ovarian, gastric and colorectal cancers (1). In order to surmount the onset of aging and cancer, complete repair of the 8-oxo-G:A mispairs is guaranteed by two different base excision repair (BER) pathways. Our previous work has revealed that the first BER event is initiated by excision of the mispaired A base by the MUTYH glycosylase followed by a long patch BER reaction, in which the DNA synthesis step is mediated by DNA polymerase (pol) λ. We have also shown that DNA pol λ, together with replication protein A (RP-A) and proliferating cell nuclear antigen (PCNA), has the unique ability among human DNA polymerases to efficiently incorporate a C opposite an 8-oxo-G, with an error frequency of 10^{-3} (2). The resulting 8-oxo-G:C pair is processed by the OGG1 glycosylase in a short patch BER reaction, leading to the removal of the 8-oxo-G lesion and the restoration of the initial G:C pair (3).
Interestingly a number of BER proteins such as NEIL1, APE1, DNA pol β, FEN1, PCNA and RP-A have been shown to physically and functionally interact with the WRN helicase/exonuclease, a protein which is mutated in Werner syndrome, an autosomal recessive disorder characterized by premature aging, cancer predisposition and genomic instability. WRN-deficient cells accumulate 8-oxo-G lesions at a much higher rate than WRN-proficient cells, suggesting that WRN plays a role in the cellular response to oxidative stress. Here, we identify WRN as a factor participating in the processing of 8-oxo-G:A mispairs in a manner dependent on DNA pol λ that catalyzes accurate DNA synthesis over 8-oxo-G. WRN physically interacts with DNA pol λ and specifically stimulates the bypass of 8-oxo-G by DNA pol λ on a one-nucleotide gap duplex resembling the DNA intermediate generated by MUTYH and APE1 during repair of 8-oxo-G:A mispairs. In cells, WRN and DNA pol λ accumulate at sites of 8-oxo-G lesions and ablation of DNA pol λ abolishes WRN recruitment to these sites. In addition, WRN and DNA pol λ appear to act in the same pathway to protect cells from the lethal effect of oxidative stress. These results suggest that WRN promotes the bypass of 8-oxo-G lesions by DNA pol λ during repair of 8-oxo-G:A mispairs. Loss of such an activity explains many phenotypes of Werner syndrome such as accumulation of oxidative DNA damage, accelerated telomere attrition, premature aging and cancer susceptibility.

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Possible role of delta-like 1 homolog (drosophila) in the development of chemoresistance in neuroblastoma

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As in other cancers, the development of chemoresistance represents a major obstacle in the successful treatment of high grade neuroblastoma (NB). In addressing the mechanisms underlying the chemoresistant phenotype in NB, we previously reported overexpression and activation of FZD1, and -catenin , hence the association of pathological activation of the wnt pathway to the resistant phenotype of NB.
In this study, a closer analysis of the gene expression profile of doxorubicin-resistant cells (LAN-1-R) compared to the parental LAN-1 cell line was performed. This analysis allowed us to identify Delta-like 1 homolog (drosophila) (DLK1) as another, moderately but significantly, overexpressed gene in the resistant variants. DLK1, a member of the Notch/delta/serrate family of proteins, is expressed in several embryonic tissues and in adult adrenal glands. DLK1 is also highly expressed in neuroendocrine tumors such as NB, suggesting a possible involvement in the development of the disease. We confirmed the increase in DLK1 expression by real-time quantitative PCR in LAN-1-R vs the non-resistant LAN-1 cells with a 5.2-fold stimulation. Higher amounts of DLK1 protein were detected on total LAN-1-R cell extracts as measured by Western blot, as well as released in resistant LAN-1-R cells culture fluid as compared to non resistant LAN-1 cells. DLK1 was overexpressed in the sensitive LAN-1 cell line showing a low DLK1 expression level, or silenced by lentiviral-mediated micro-adapted shRNA, in LAN-1-R cell line. Overexpression of DLK1 in the LAN-1 cell line highly influenced the proliferative behaviour of these cells without modifying their drug sensitivity. In contrast, silencing of DLK1 in the LAN-1-R cell line restored caspase-3 activation upon different drug treatment, suggesting a role of DLK1 in the drug resistant phenotype of the LAN-1-R cell line.
Our data which fully support a recent report, implicating DLK1 in enhanced tumorigenic and undifferentiated characteristics of NB cells, further propose a role for DLK1 in their multi-drug resistant phenotype. These observations which associate DLK1 to multiple mechanisms leading to the particularly malignant behaviour of NB, deserve further investigation.

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Phosphorylation-dependent regulation of a specific USP7 isoform controls Mdm2 and p53 levels in response to DNA damage

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USP7 (also known as HAUSP or herpes virus-associated ubiquitin-specific protease) plays an important role in the DNA damage response by controlling cellular levels of the p53 tumour suppressor protein, a transcription factor that regulates cell cycle progression and apoptosis following DNA damage and is also critical for the regulation of cellular senescence and tumour suppression. In unstressed cells, p53 is normally expressed at a low level since it is controlled by ubiquitylation-dependent proteasomal degradation by the E3 ubiquitin ligase Mdm2. However, self-ubiquitylation of Mdm2 promotes its own degradation which is rescued by formation of a complex between Mdm2 and USP7, highlighting an important role for the regulation of the cellular levels of these proteins in p53 stability and activity. It was recently reported that USP7 is phosphorylated at serine 18 (Ser18) that may play an important role in the function of the enzyme, and therefore may affect the p53-dependent response to DNA damage. However the physiological role of this phosphorylation, as well as the protein kinase and phosphatase involved in the regulation of USP7 phosphorylation status, was unknown.
Here, we report that a specific isoform of USP7 plays a major role in the stabilisation of Mdm2 and consequently controls degradation of the p53 protein. We show that cellular activity and stability of this USP7 isoform is regulated by phosphorylation at Ser18 by casein kinase 2 and dephosphorylation by protein phosphatase 1G (PPM1G). In addition, we provide evidence that PPM1G is activated in response to DNA damage in an ATM-dependent manner, which leads to extensive USP7 dephosphorylation, and to destabilization of Mdm2 and consequently an up-regulation of p53. Collectively, these results provide new mechanisms by which USP7 regulates the p53-dependent cellular response to DNA damage.

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Extent and patterns of O6-methylguanine methyl transferase (MGMT) promoter methylation in glioblastoma and respective derived spheres. What matters?

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Glioblastoma (GBM) is the most common and most malignant form of primary brain tumors in adults. Epigenetic silencing of the MGMT gene by promoter hypermethylation blunts repair of O6-methyl guanine (1) and has been shown to be a predictive factor for benefit from alkylating agent therapy in GBM patients (2). Consequently clinical trials now stratify or select glioblastoma patients according to the MGMT methylation status. However, the best technique of MGMT status determination, cutoff definition between methylated and unmethylated, and the relevance of extent of methylation are all subject to ongoing controversy. Quantitative methylation specific tests suggest that not all cells in a glioblastoma with detectable promoter methylation of the MGMT gene carry a methylated MGMT allele (3). This observation may indicate cell subpopulations with distinct MGMT status, raising the question of the clinically relevant cut-off of MGMT methylation therapy. In order to address this question, ten paired samples of glioblastoma and respective GBM-derived spheres (GS), cultured under stem cell conditions, were analyzed for the degree and pattern of MGMT promoter methylation by methylation specific clone sequencing, MGMT gene dosage, chromatin status, and respective effects on MGMT expression and MGMT activity.
In GBM MGMT methylated alleles ranged from 10 to 90%. In contrast, methylated alleles were highly enriched (100% of clones) in respective GS. The CpG methylation patterns were characteristic for each glioblastoma exhibiting 25 to 90% methylated CpGs of 28 sites interrogated. Furthermore, MGMT promoter methylation was associated with a non-permissive chromatin status in accordance with very low MGMT transcript levels and undetectable MGMT activity
In MGMT methylated GBM, MGMT promoter methylation is highly enriched in GS, which supposedly comprise glioma initiating cells. Thus, even a low percentage of MGMT methylation measured in a GBM sample may be relevant and predict benefit from an alkylating agent therapy.

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Y-Radiation enhances immunogenicity of cancer cells by increasing the expression of cancer-testis antigens in vitro and in vivo

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Radiotherapy is an effective treatment for cancer. Irradiation induces apoptosis of tumor cells, but there is accumulating evidence that it also supports adaptive immunity. It was described that irradiation induces de novo protein synthesis and enhances antigen presentation. Because the immune system often recognizes the tumor, we hypothesized that radiotherapy up-regulates the expression of cancer-testis (CT)-antigens and MHC class I, thus making the tumor more immunogenic. We found that irradiation of cancer cell lines induced higher or *de novo* expression of different CT-antigens and up-regulated MHC class I expression in a time- and dose-dependent fashion. We confirmed these results with fresh tumor biopsies that were *ex vivo* irradiated and with paired samples from sarcoma patients taken before and after radiotherapy. Our findings suggest that Y-radiation makes tumors more immunogenic and that the combination of radiotherapy plus immunization with relevant CT-antigens or blockade of co-inhibitory interactions or mediators or the stimulation of innate immune cells may be an effective treatment for cancer patients.

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The Wnt inhibitory factor 1 (WIF-1) has tumor suppressing functions in glioblastoma by inducing cellular senescence

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Glioblastoma is the most aggressive form of human glioma and despite recent progress in therapy, the prognosis remains dismal with a median survival of 15 months. Expression based prediction of gene alterations identified Wnt inhibitory factor I (WIF1) as a new candidate tumor suppressor gene involved in glioblastoma. WIF1 encodes a secreted Wnt antagonist and it was strongly down-regulated in most glioblastoma as compared to normal brain, implying deregulation of Wnt signaling. Since Wnt signaling is involved in diverse cellular processes, Wnt pathway deregulation can dramatically alter cell growth, differentiation and cell fate decisions. In glioblastoma, silencing of the WIF1 gene was found to be mediated by deletion and WIF1 promoter hypermethylation. Re-expression of WIF1 in glioblastoma cell lines revealed a dose dependent decrease of Wnt pathway activity. To further dissect the biological effects of WIF1 expression, we established WIF1 overexpressing glioblastoma cell lines. We observed that WIF1 re-expression inhibited cell proliferation *in vitro* and strongly reduced anchorage independent growth. The ability of forming colonies in soft agar was reduced to 11% of the control. Moreover, the expression of WIF1 was able to completely abolish tumorigenicity in a respective xenograft model in nude mice. Interestingly, WIF1 overexpression in glioblastoma cells induced a senescence-like phenotype characterized by the appearance of enlarged flattened and multinucleated cells positive for the presence of β-galactosidase, a late marker of senescence. These results provide evidence that WIF1 has tumor suppressing properties in glioblastoma, hence, the implication of a deregulated Wnt pathway may render glioblastoma sensitive to Wnt signaling inhibitors, potentially by diverting the tumor cells into a senescence-like state.

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The role of ATM-mediated CtIP phosphorylation in DNA double-strand break processing

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Maintenance of genome stability depends on efficient and accurate repair of DNA damage. DNA double-strand breaks (DSBs) are among the most lethal types of DNA damage, with the potential to cause chromosomal rearrangement and thereby triggering carcinogenesis. DSBs are induced by ionizing radiation or certain chemotherapeutic drugs, but can also arise through perturbations in DNA replication. Nonhomologous end-joining (NHEJ) and homologous recombination (HR) are the predominant DSB repair pathways. A major difference between these pathways is that HR is initiated by extensive DNA end resection, while NHEJ requires only limited DSB processing. We have recently identified the human CtIP protein to play a key role in DNA end resection and HR (1).
The cellular response to DSBs involves a protein phosphorylation cascade propagated through DNA damage-activated kinases, most importantly ATM. Through phosphorylation at consensus SQ/TQ motifs, ATM activates many DNA damage response components involved in cell cycle arrest, DNA repair and apoptosis. Furthermore, efficient DNA resection has been shown to require ATM kinase activity, but the mechanistic link between ATM-mediated phosphorylation events and DSB processing still remains elusive. CtIP was shown to become phosphorylated at three distinct sites in an ATM-dependent manner upon treatment of cells with DSB-inducing agents. However, the role of ATM-dependent CtIP phosphorylation is unknown.
In order to adress this question we generated U2OS cell lines stably expressing siRNA resistant GFP-tagged CtIP wild-type and two CtIP mutants, where all three putative ATM phosphorylation sites were substituted with either non-phosphorylatable alanine residues (3SA) or phospho-mimicking glutamic acid residues (3SE). We could show that both CtIP mutants localize to damaged chromatin. At the moment we are conducting live cell imaging experiments of microlaser-irradiated GFP-CtIP cells to investigate whether CtIP phosphorylation has an influence on the kinetics of recruitment to DSBs. In parallel, we isolated chromatin from all three cell lines before and after treatment with genotoxic agents to examine whether ATM phosphorylation has a global impact on CtIP chromatin association/dissociation. Finally, we are performing clonogenic survival assays in all three cell lines treated with various DNA damaging agents.
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A genome-wide screen for renal cancer genes by targeted transposon-mediated mutagenesis in vivo

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„Sleeping Beauty" is a novel and elegant method for the genome-wide identification of cancer genes (1). By somatic activation of oncogenic transposons, insertional mutagenesis causes tumor formation in transgenic mice. Integration occurs at random but cancer-causing insertions are preferably amplified in tumors by clonal selection. Based on the known and invariant DNA sequence of the transposon the integration sites can be directly am-

plified by ligation-mediated PCR. Sequencing, then, identifies the genomic DNA immediately flanking the integration site. This leads to the rapid identification of affected genes (2).
Using proprietary Pax8-rtTA mice (3), which allow for efficient kidney-specific modulation of transgene expression, we have adapted Sleeping Beauty for specific use in renal cancer. Six transgenes (Pax8-rtTA, LC1, tet-o-cMYC, p53flox, Rosa-SBflox, T2onc2) were combined which allowed us to simultaneously
- overexpress c-myc
- knockout p53
- and activate Sleeping Beauty
in the kidneys of transgenic mice. This strategy allowed us to enforce tumor development in the kidney with regard to both time of onset and multiplicity of tumors. We have amplified and sequenced the transposon integration sites of ≈100 mouse renal cell carcinomas derived from this model. We have established a total number of 275 000 single reads (unique integrations plus replica) among them 75 000 unique hits in the mouse genome. The integration sites were evenly distributed among all chromosomes and we did not observe a statistically significant number of integrations in either the c-myc or the p53 locus. Since both genes were already integral parts of our model these findings were entirely as expected. We found, however, statistically significant hits (non-random integration) in a total number of 120 genes ($10^{-100} < p < 10^{-5}$), among them Pten, a well-recognized renal tumor suppressor gene also in human renal cancer. Ongoing work aims at evaluating the functional role of the 120 candidate genes in human renal cancer. Our findings demonstrate that Sleeping Beauty can be successfully used to identify primary renal cancer genes with relevance to human cancer.

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Bimodal mechanism of DNA repair stimulation by UV-damaged DNA binding protein 2 in chromatin

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Mammalian cells are in need of the UV-damaged DNA binding (UV-DDB) protein, a heterodimer of DDB1 and DDB2, for UV-lesion recognition in the global genomic nucleotide excision repair pathway. The DDB1 subunit is an adaptor that associates with CUL4A ubiquitin ligases, whereas DDB2 binds avidly to UV-irradiated chromatin. But, until now, inconsistent data have been reported as to how this factor stimulates DNA damage excision. In the present report, the so far enigmatic link between DNA repair, DDB1-DDB2, XPC and the CUL4A ligase has been elucidated by chromatin partitioning, in situ mapping of interaction domains and live-cell imaging of protein dynamics. This study describes DDB2 as a polyvalent DNA repair organizer in the physiological chromatin context. First, we demonstrate a novel ubiquitin-dependent function whereby DDB2 sorts out DNA repair hotspots, characterised by high accessibility for downstream nucleotide excision repair factors and MNase-sensitivity, for a very fast initial recruitment of the XPC partner. By mediating its ubiquitylation, DDB2 optimizes the spatiotemporal chromatin distribution of XPC, thus preventing the futile migration of this repair initiator to compacted, MNase-resistant nucleosomes that are refractory to downstream excision complexes. In the long term and by interacting directly with its DNA-binding domain, DDB2 recruits XPC to UV lesions anywhere in chromatin and, finally, promotes a thermodynamically unfavorable β-hairpin insertion into the double helix.

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RAD50 and HELQ-novel insights into the repair of DNA double-strand breaks

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DNA double-strand breaks (DSBs) are one of the most critical lesions with respect to survival and preservation of genomic integrity. A key role in recognizing, signaling and repair of DSBs is ascribed to the MRE11-RAD50-NBS1 (MRN) complex. Human CtIP associates with the MRN complex to initiate the resection of DSBs that is required for the repair by homologous recombination (HR) (1). It is evident, that the generation of those highly recombinogenic single-stranded DNA structures have to be controlled in a cell-cycle-dependent manner. In addition, CtIP and MRN are implicated in other DNA metabolic processes than recombination, such as DNA replication and cell-cycle checkpoints. It is widely accepted that such diverse functions of single polypeptides are mainly regulated by either protein-protein interactions or by post-translational modifications.
As a first project, we would like to gain further mechanistic insights into how RAD50S (for separation-of-function) mutations affect genome stability. We speculate that some of the phenotypes previously reported for RAD50S mutant yeast and mice are attributed to alterations in the MRN-CtIP complex formation (2). For this purpose, we have complemented an immortalized RAD50-deficient patient cell line with either wild-type human RAD50, or two RAD50S mutant alleles bearing single amino-acid substitutions (K22M and R83I) by using retroviral constructs.
Furthermore, we have recently identified HELQ (alternative name: HEL308), a 3'-to-5' DNA helicase with RecQ-like properties, in a proteomic screen for DNA damage-specific interaction partners of CtIP. There are some indications in the literature that HELQ is implicated in the repair of interstrand crosslinks, however very little is known about its function in human cells (3). Our current studies are focused on elucidating the role of HELQ in DSB repair and its connection to CtIP.
In summary, both projects are aimed to get a more detailed understanding on how cells sense and repair DSBs in order to maintain genome integrity.

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Src regulates Inhibitor of differentiation 1 (ID1) via c-MYC induced repression of microRNA-29b: Implications for invasion of lung cancer cells and their resistance against Src inhibitors

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The c-Src kinase regulates cancer cell invasion through ID1. ID1 is frequently overexpressed in primary human lung adenocarcinoma, accompanied by overexpression of Src and MMP9.(1) We previously demonstrated that Src regulates ID1 in human lung cancer cells.(2) MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression by inducing translational inhibition or cleavage of their target mRNAs. The Myc proto-oncogene is able to suppress the expression of several miRNAs, including the members of the miR-29 family.(3) The current study aimed at identifying miRNAs involved in the Src-ID1 signaling in lung cancer, and to test if modulation of these miRNAs and ID1 in non small-cell lung cancer cells leads to resistance against Src inhibitors. Upon incubation of A549 lung cancer cells with the Src inhibitor saracatinib, miR-29b was one of the most highly upregulated miRNAs in a miRNA array with predicted binding to the ID1 3'UTR. Indeed, ID1 3'UTR luciferase reporter assays revealed direct binding of miR-29b to the ID1 3'UTR, but not to a mutated ID1 3'UTR. Src inhibition with either saracatinib or dasatinib led to a dose-dependent increase of miR-29b and a decrease of ID1 in different lung cancer cell lines (A549, H460, H1299, H820, HCC827). Anti-miR-29b enhanced ID1 and MMP9 mRNA and protein levels. Migration – measured with a wound healing assay – as well as invasion – determined by modified Boyden Chambers with mouse-matrigel-coated filter inserts with 8.0 µm pores – were both significantly increased by anti-miR-29b. In contrast, transfection with pre-miR-29b suppressed the level of ID1 and significantly reduced cell migration and invasion, a hallmark of the Src-ID1 pathway. Anti-miR-29b and ID1 overexpression diminished the effects of saracatinib and dasatinib on migration and invasion. Saracatinib and

dasatinib decreased c-Myc and Id1 protein levels, whereas forced expression of c-MYC repressed miR-29b and induced ID1. miR-29b was significantly downregulated in 23 formalin-fixed and paraffin-embedded adenocarcinoma samples compared with neighboring alveolar lung tissue, and miR-29b expression was inversely correlated with poor prognosis (event-free and overall survival). These results suggest that miR-29b is involved in the Src-ID1 signaling pathway. In lung adenocarcinoma miR-29b is dysregulated and a prognostic factor. Furthermore, miR-29b is a potential predictive marker for Src kinase inhibitors.

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Investigating the role of CtIP phosphorylation in the DNA damage response and in cancer

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Human CtIP is a nuclear phosphoprotein and a multivalent adaptor connecting transcriptional regulation, checkpoint control and tumor suppression. For instance, CtIP together with its binding partner CtBP can co-repress the expression of several pro-apoptotic genes. Moreover, CtIP controls G1/S cell cycle progression by modulating cyclin D1 expression. More recently, CtIP was shown to be involved in the DNA damage response by initiating DNA end resection, which is required for the repair of DNA double-strand breaks by homologous recombination.
Human CtIP contains various conserved, potential phosphorylation sites. However, very little is known about which of these sites are phosphorylated in vivo, by which protein kinases, and what is the biological relevance of these phosphorylation events.
We recently identified several novel phosphorylation sites and some phosphorylation-dependent interaction partners of CtIP. One of them is the peptidyl-prolyl cis/trans isomerase PIN1. PIN1 specifically binds phosphorylated serine (S) and threonine (T) residues (in a S/TP context) to induce a cis/trans conformational switch within the target protein. In this way PIN1 was shown to control diverse processes such as protein localization, activity and/or stability. Our preliminary data indicate that PIN1 binds CtIP in a phospho-dependent manner. Furthermore, we could identify two conserved S/TP sites of CtIP responsible for PIN1 binding. By NMR-spectroscopy we could show that PIN1 can induce a cis/trans conformational change within a synthetic CtIP-phosphopeptide corresponding to the PIN1 binding site. Our preliminary in vivo data suggests that the cis/trans conformational change of CtIP triggered by PIN1 is responsible for CtIP protein turnover. At the moment phenotypic analysis of cells expressing PIN1 binding mutants of CtIP are carried out. We speculate that alterations in the control of CtIP protein turnover can affect cellular transformation capacity but may also influence the cellular responses to chemotherapeutic agents.

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GAA repeats induce post-replicative DNA junctions in mammalian cells

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Background/Hypothesis: Trinucleotide repeats (TNR) are members of the microsatellite family. They can undergo large deletions or expansions, leading to neurodegenerative diseases like Friedreich's ataxia, Huntington disease or Fragile X. Expanded TNRs were shown to form stable secondary structures in bacteria and to stall replication forks in yeast. However, the molecular mechanisms leading in vivo to repeat expansion and replication interference are still elusive. The aim of this project is to gain insight into the DNA structures and cellular factors mediating TNR instability in vivo in human cells.
Methods: We established an experimental system to analyse DNA replication intermediates (RI) across TNR in human cells. SV40-derived plasmids containing GAA tracts in different number and orientation are transfected into 293T or U2OS cells and allowed to replicate. Plasmid RI are recovered and analyzed by a combination of bidimensional (2D)-gels, psoralen crosslinking

and electron microscopy (EM).
Results: We observe that GAA repeats induce a transient fork pausing in a length- and orientation-dependent manner, without affecting the location of replication termination. Our 2D-gels reveal several unexpected TNR-dependent signals that are currently under extensive investigation. In particular, distinctive X shaped molecules accumulate at fully replicated plasmids containing long GAA repeats. These molecules can be distinguished from recombination or termination intermediates and may derive from pre-existing junctions observed at replicating plasmids. By extraction of these molecules from the 2D-gels and direct visualization at the EM we are currently investigating the structural determinants of both control and GAA-specific intermediates.
Conclusions: Our studies allowed the identification of alternative DNA structures associated with expanded GAA repeats, which we are further characterizing by direct EM observation. Combining these approaches with depletion (siRNA) of specific factors, we aim to reveal the role of candidate cellular players in TNR replication and stability.

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Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib – A Phase II trial

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Amplification of the EGFR is one of the most common oncogenic genetic alterations in primary glioblastoma making it a prime target for therapy. However, clinical trials in glioblastoma testing small molecule inhibitors of the EGFR tyrosine kinase failed, although some responses were observed. Here we aimed at investigating the molecular effects of preoperative treatment with the tyrosine kinase inhibitor Gefitinib on the EGFR signalling pathway in glioblastoma of patients enrolled in a clinical trial (HUwww.clinicaltrials.govUH , NCT00250887).
Patients selected for re-operation of a glioblastoma were treated at least 5 days with 500 mg Gefitinib prior to surgery, followed by post-operative Gefitinib until recurrence. Resected glioblastoma tissues exhibited high concentrations of Gefitinib (median, 4100 ng/g tumor tissue) that were more than 20 times higher than in the plasma (median, 181 ng/ml plasma). The frequency of EGFR amplification in the patient cohort was 7/22. The molecular analysis revealed that the EGFR was efficiently dephosphorylated in the treated patients as compared to a control cohort of 12 patients (7/12 with EGFR amplification), while downstream pathway constituents did not seem to be affected by the treatment as determined by semi-quantitative, phosphorylation specific assays (Bioplex, Biorad). In contrast, *in vitro* treatment of a glioblastoma cell line, BS153, with endogenous EGFRwt amplification and EGFRvIII expression resulted not only in dephosphorylation of the EGFR, but also of key regulators in the pathway like p-AKT. Intriguingly, a respective in vivo model, treating nude mice with established subcutaneous BS153 xenografts (1cm diameter) using the human dosing scheme over 5 days, showed dephosphorylation of the EGFR, however, similar to the human glioblastoma, downstream constituents of the pathway were not affected.
In conclusion, Gefitinib reaches high concentrations in the tumor tissue and efficiently dephosphorylates its target. However, regulation of downstream constituents in the EGFR pathway seems to be dominated by EGFR phosphorylation independent regulatory circuits. Beside negative feed-back loops of the pathway other pathophysiologic signals such as hypoxia may contribute to this effect under steady state conditions in vivo. Thus, additional rate limiting constituents of the pathways influencing EGFR downstream signalling need to be targeted for effective glioblastoma treatment.

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Replication fork regression precedes double strand break formation upon topoisomerase I inhibition

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Topoisomerase I (Top1) activity needs to be tightly regulated in every unperturbed cell cycle to ensure proper DNA replication fork progression and avoid DNA damage. Top1 can be trapped by specific inhibitors, such as camptothecin (CPT) and its derivatives, leading to interference with DNA metabolism and resulting in potent cytotoxicity exploited for cancer chemotherapy. Although replication-induced double strand breaks (DSB) have been consistently proposed to mediate this cytotoxicity, recent reports invoked increased topological tension upon Top1-inhibition as a possible alternative source of genotoxic stress.
In this work, combining molecular biology techniques and visualization of replication intermediates by electron microscopy, we structurally investigated the impact of Top1 poisoning on DNA replication, exploiting different technical benefits of three eukaryotic systems - *S. cerevisiae*, human cell culture and *Xenopus egg* extracts. We provide direct *in vivo* evidence in all systems that Top1 inhibition rapidly slows down replication fork progression and induces fork regression, resulting from topological stress accumulation. Both phenomena can be linked to chromosomal locations susceptible to accumulate topological stress and can be uncoupled from DSB formation. Consistently, homologous recombination-mediated DSB processing does not contribute to bulk DNA replication upon Top1 inhibition and is not required to trigger the DNA damage checkpoints at low CPT doses. We propose that fork regression is a frequent and early event in response to CPT-mediated increase in DNA supercoiling and could elicit per se activation of the DNA damage response. We are currently exploring cellular pathways mediating fork reversal and processing reversed forks, finally leading to the observed replicative DSB upon Top1 inhibition.

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Strand-selective and site-specific DNA lesion demarcation by the xeroderma pigmentosum group D helicase

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The most detrimental reactions of the UV-exposed skin, including cutaneous erythema, hyperplasia and cancer, are triggered by cyclobutane pyrimidine dimers (CPDs). Although placental mammals depend on nucleotide excision repair (NER) to eliminate CPDs, none of the core NER factors is able to distinguish this highly mutagenic lesion from native DNA, raising the question of how CPDs are recognized to define the correct DNA repair boundaries. A key intermediate in the NER pathway involves unwinding of the damaged duplex by transcription factor TFIIH through a helicase reaction that is mediated by xeroderma pigmentosum group D (XPD) protein. In live-cell imaging studies, we observed that the enzymatic activity of XPD induces the anchoring of this TFIIH subunit to UV lesion sites in mammalian nuclei. This unique interplay of the XPD helicase with damaged DNA has been analyzed using a monomeric archaeal homolog, which demonstrates that the collision with a single CPD inhibits the helicase but stimulates its ATPase activity. Restriction digestion and glycosylase protection assays show that the XPD helicase remains firmly bound to a CPD situated in the translocated strand along which the enzyme moves with 5′-3′ polarity. Competition assays confirm that a stable nucleoprotein intermediate is formed when the XPD helicase encounters a CPD in the translocated strand. Instead, the enzyme readily dissociates from the substrate after running into a CPD located in the displaced 3′-5′ strand. These results reveal a dynamic DNA damage verification process that culminates in strand-selective and site-specific lesion demarcation by the XPD helicase.

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Genetic pathways to WHO grade II low-grade gliomas

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To establish genetic profiles for diffuse gliomas and to estimate their predictive impact, we screened 360 WHO grade II gliomas for mutations in the IDH1, IDH2, and TP53 genes and for 1p/19q loss and correlated these with clinical outcome. Most tumors (86%) were characterized genetically by TP53 mutation plus IDH1/2 mutation (32%), 1p/19q loss plus IDH1/2 mutation (37%), or IDH1/2 mutation only (17%). TP53 mutations only or 1p/19q loss only was rare (2% and 3%). The median survival of patients with TP53 mutation with or without IDH1/2 mutation was significantly shorter than that of patients with 1p/19q loss with or without IDH1/2 mutation (51.8 months vs 58.7 months; P=0.0037). Multivariate analysis with adjustment for age and treatment confirmed these results (P=0.0087), and also revealed that TP53 mutation is a significant prognostic marker for shorter survival (P=0.0005) and 1p/19q loss for longer survival (P=0.0002), while IDH1/2 mutations are not prognostic (P=0.8737). Thus, the molecular classification on the basis of IDH1/2 mutation, TP53 mutation and 1p/19q loss has power similar to histological classification of diffuse gliomas. Since this is more objective than histological typing and correlates well with clinical outcome, molecular profiling complements histological typing, particularly of oligoastrocytomas. Only 26 tumors (7%) were triple-negative (i.e. lacking any of these alterations), suggesting that there may be an alternative genetic pathway for the development of low-grade diffuse glioma. In the present study, array comparative genomic hybridization in 15 triple-negative WHO grade II gliomas (8 diffuse astrocytomas and 7 oligodendrogliomas) showed loss at 9p21 (p14ARF, p15INK4b, p16INK4a loci) and 13q14–13q32 (containing the RB1 locus) in 3 cases and 2 cases, respectively. Further analyses in 31 triple-negative cases as well as a total of 160 non-triple-negative cases revealed that alterations in the RB1 pathway (homozygous deletion and promoter methylation of the p15INK4b, p16INK4a, and RB1 genes) were significantly more frequent in triple-negative (26%) than in non-triple-negative cases (11%; P=0.0371). Alterations in the RB1 pathway in triple-negative cases were significantly associated with unfavorable patient outcome. These results suggest that a fraction of low-grade diffuse gliomas lacking common genetic alterations (IDH1/2 mutation, TP53 mutation and 1p/19q loss) may develop through a distinct genetic pathway, which includes loss of cell-cycle control regulated by the RB1 pathway.

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In vitro inhibiton of KIT signaling by Nilotinib is regulated via the PI3K/AKT pathway in acrolentiginous melanoma cell cultures

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Mitogen-activated protein kinase (MAPK) and protein kinase B (AKT) are considered to be principal pathways transferring tumorigenic KIT signals in Acrolentiginous (ALM) and mucosal melanoma (MM). Several clinical case reports demonstrate the efficacy of KIT tyrosine kinase receptor as a drug target inducing tumour regression in vivo[1-3]. KIT status was analysed for 25 ALM and 5 MM cell cultures. Extracellular signal-regulated kinase (ERK) and Phosphoinositide-3-kinase (PI3K) expression and activity were studied 72 hours subsequent to Nilotinib treatment in 2 ALM and 2 MM cell cultures. No KIT mutation was detected, but one cell hosted the BRAF V600E mutation.

Nilotinib inhibited cell proliferation in one, KIT wildtpye acrolentiginous metastatic melanoma cell culture out of 25. ERK and phospho-ERK expression levels were not affected by Nilotinib treatment. Phospho-KIT (703 and 719), total KIT and PI3K subunits alpha, beta and gamma were not expressed in three cell cultures. However, the responding cell culture revealed decreasing total KIT and PI3K delta expression intensities corresponding to increasing Nilotinib concentrations. Melan A expression was detected in equal distribution in the Nilotinib responding cell culture only.

Nilotinib inhibits cell proliferation of an ALM despite wildtype KIT. KIT targeted growth inhibition interferes with the PI3K pathway, independent of the MAPK signaling pathway in this melanoma subtype.

Larger studies are needed to further understand this mechanism, including the relevance of KIT mutations for tumor progression *in vitro* and *in vivo*.

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Characterization of a novel anti-apoptotic signaling pathway downstream of FGFR4 in Rhabdomyosarcoma

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FGFR4 is highly overexpressed in rhabdomyosarcoma tumors and contains activating mutations in eight percent of the cases (1-2). These data strongly suggest that FGFR4 plays an important role in the tumorigenesis of this cancer type. However, the molecular mechanisms behind the benefit of active FGFR4 for rhabdomyosarcoma cells are largely unknown.
Here, we describe a survival pathway activated by FGFR4 in Rhabdomyosarcoma cells. Active FGFR4 is able to block apoptosis induced by inhibition of other central signaling pathways with small molecule inhibitors, such as the IGF1R pathway with AEW541 and PI3K-controlled pathways with BEZ235. Using different biochemical and genetic approaches, we could locate the apoptotic block upstream of the pro-apoptotic BH3-only proteins bmf and bim. To further elucidate the mechanism of this prosurvival signaling, we then performed proteomic studies to define the complete interactome of FGFR4 in Rhabdomyosarcoma cells. These analyses revealed a strong interaction of FGFR4 with PLCγ, linking FGFR signaling to PKCs. Indeed, activation of PKCs strongly inhibits AEW541/BEZ253-induced apoptosis, suggesting that PKCs are central for FGFR4-activated survival signaling. In contrast, the classical survival pathways via AKT and ERK are not or only marginally involved in the rescue mechanism.
Treatment approaches targeting the IGF1R/PI3K axis in rhabdomyosarcoma are under intensive investigation. Our data suggest that rescue mechanisms via FGFR4 and PKCs should be taken into account when applying such treatment strategies. A combination of IGF1R- and FGFR4/PKC-inhibitors therefore might be superior compared to single treatments.
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DNA polymerase switch on an 8-oxo-G lesion from a replicative to a translesion synthesis DNA polymerase

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Cells are perpetually exposed to reactive oxygen species (ROS), which derive either from exogenous (e.g. smoking, pollution and UV) or endogenous sources (e.g. by-products of cellular respiration and components of the inflammatory responses). When the balance between oxidants and antioxidants is disrupted, the cells are in an "oxidative stress". One of the consequences of this stress, is the formation of DNA lesions, including 7,8-dihydro-8-oxoguanine (8-oxo-G), which often results in C:G->T:A transversion mutations. These mutations can be the source of cellular transformation and eventually lead to different cancers (1). Cells implement many different pathways to repair lesions as exemplified by DNA polymerase (pol) switches from replicative to repair and/or translesion synthesis (TLS) pols. The aim of this project is to analyse the switch from the replicative pol δ to the 8-oxo-G TLS pol λ. As shown before in our laboratory, when the damage is not removed before DNA replication, pol δ incorporates with high frequency an inaccurate A opposite 8-oxo-G and creates a A:8-oxo-G mismatch (2). After the removal of this A by the MUTYH DNA glycosylase and AP endonuclease 1, pol λ incorporates a correct C opposite 8-oxo-G in the presence of replication protein A and proliferating cell nuclear antigen (PCNA) (3). By using different primer extension and gap-filling assays containing an 8-oxo-G lesion, pol δ is blocked at the nucleotide preceding the lesion, but can incorporate preferentially a C opposite 8-oxo-G in the presence of PCNA. When pol λ is added, it assists pol δ to extend from this lesion. In addition, the size of the downstream gap after the lesion seems to be important for the TLS action of pol λ. A gap size between 2 and 6 nucleotides seems to be optimal for the TLS by pol λ. Our results suggest that a switch appears to exist between pol δ and pol λ on the 8-oxo-G lesion.

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Regulation of MutY Homolog DNA Glycosylase (MYH)

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A high amount of reactive oxygen species can lead to oxidative stress in human and animal cells, which has been shown to account for most spontaneous DNA damage. One of the best characterized indicators of such damage in DNA is the generation of 8-oxo-G. Opposite this lesion, A is frequently incorporated by replicative DNA polymerases (pols), which, if not repaired, leads subsequently to G:C -> T:A transversion mutations. This transversion is the most prevalent somatic variation occurring in different types of cancer (1). To counteract this, the base-excision-repair (BER) pathway is initiated by action of DNA glycosylase MYH, which excises mispaired adenine opposite the lesion. Germline mutations in MYH are correlated with a predisposition for the development of MYH-associated polyposis (MAP), which is characterized by the formation of multiple adenomas. For the subsequent repair, a specialized pol is required to fill the gap correctly. We showed that pol λ is able to perform correct bypass over an 8-oxo-G lesion (2). Recently we found a direct interaction between pol λ and MYH, suggesting a role for MYH in the accurate 8-oxo-G repair pathway (3). The interaction site of both enzymes was identified using GST-pulldown. Although an important role for MYH in cancer development is already demonstrated, very little is known about its regulation and posttranslational modifications. To address these questions stimulation of MYH by oxidative stress will be analyzed as well as an influence of E3-ligase Mule, which was recently shown to regulate degradation of BER members, on protein stability.

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MicoRNA-mediated ATM-depletion leads to a pronounce DNA repair defect and clinically manifest tumor radiosensitivity

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Head and neck tumors are generally characterized by a moderate radiosensitivity. From our lab, we reported a tumor case (SKX, SCC oral cavity) with an extreme radiosensitivity that had been cured by moderate radiotherapy protocol without further need for surgery. Both SKX cells and xenograft showed a pronounced repair defect with a high number of residual -H2AX foci 24h after irradiation. Protein expression of KU70, KU80, DNAPKcs, LIGIV, XRCC4, Artemis and RAD51 as well as DNA-PK kinase activity were normal. Importantly, we show an ATM-deficiency as SKX cells express no ATM protein and show impaired ATM damage signaling (absence of p-ATM, p-SMC1, p-Chk2, p-Kap1) after irradiation. Sequencing of the 66 exons of ATM gene revealed no somatic or splicing mutations in SKX cells. Real time PCR show 3-fold lower ATM mRNA level in SKX compared to FaDu cells. We found a moderate ATM promoter hypermethylation that could be reverted by treating SKX cells with 5-aza-2'-deoxycytidine; however no ATM protein was detected. Instead, we demonstrated a post-transcriptional regulation of ATM in SKX cells via 6-fold enhanced miR421 level (compared to reference FaDu cells) that targets the 3'-UTR of ATM mRNA. Transfection of anti-miR421 inhibitor or microRNA-nonsensitive ATM expressing vector recovered ATM expression and strikingly reduced the hyper-radiosensitivity of SKX cells. This is the first report about ATM regulation via microRNA in a human malignancy that leads to clinically manifest tumor radiosensitivity.

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Mismatch repair (MMR) deficiency: A temozolomide resistance factor in medulloblastoma cell lines that is uncommon in primary medulloblastoma tumors

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With temozolomide (TMZ) potentially becoming a standard chemotherapeutic agent in the clinical management of recurrent childhood medulloblastoma (MB), we aimed to examine: 1) whether the status of MMR genes influences the sensitivity of MB cells toward TMZ, and 2) the clinical relevance of the *in vitro* results by surveying the expression levels of MMR genes among 60 pediatric primary MB samples. The anti-proliferative effect of TMZ on MB cells was examined *in vitro* using the clonogenic survival assay. The expression of all four MMR genes (MLH1, MSH2, MSH6, PMS2) were analyzed by western blot analysis (for MB cell lines DAOY and D341Med; D425Med in another report (1)), and Affymetrix U133 plus2 expression array (14 MB cell lines and 60 primary MB tumors). Additionally, DNA sequencing and promoter methylation analysis were employed to further characterize the MLH1 gene in D341Med cells. TMZ exhibited potent anti-proliferative activity in MGMT-deficient D425Med cells and only moderate activity against MGMT-proficient DAOY and D341Med cell lines. We recently found that the addition of MGMT inhibitor O6-benzylguanine (O6-BG) increased the potency of TMZ in DAOY, but not in D341Med cells (2). Both these cell lines are MGMT-proficient (2). Discrepancy in TMZ response is explained by the consideration that DAOY has a functioning MMR system (as suggested by the expression of MLH1, MSH2, PMS2 and MSH6 genes), while D341Med is MMR-deficient due to transcriptional silencing of MLH1. Expression array analysis, indeed identified 4 (D341Med cells and 3 additional cell lines) out of 14 MB cell lines that do not express MLH1. However, transcriptional profiling also indicated that these MMR genes are normally expressed in 60 primary MB tumors, which is consistent with a smaller study involving 22 primary MB tumors (3). In summary, our analyses suggest that although MMR deficiency (in addition to elevated MGMT activity) can reduce the anti-tumor activity of TMZ, this may not be a major clinical concern since such deficiency is uncommon in primary MB tumors.

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The EWS/FLI1 oncogenic transcription factor inhibits expression of PHLDA1

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The Ewing's sarcoma family of tumors is a group of malignancies affecting bone and soft tissue in childhood and adolescence. The biology of these very aggressive osteolytic tumors revolves around EWS/FLI1, a chimeric transcription factor that is considered to be a hallmark of Ewing's sarcoma. EWS/FLI1 plays quintessential role in the pathogenesis of Ewing's tumors by deregulating gene expression. Therefore, this study is focused on identification of physiologically important target genes. We performed a meta-analysis of published gene expression profiles from 3 Ewing's cell lines in order to exclude cell type dependent results. Additionally, we compared expression profiles of other small-round-blue-cell tumors with expression profiles of Ewing's tumors. Finally, we generated a list of candidate EWS/FLI1 target genes whose expression correlated best with EWS/FLI1 expression levels in the entire range of cells tested by down-regulation of endogenous EWS/FLI1. The gene exhibiting most marked response upon silencing of EWS/FLI1 was PHLDA1 (Pleckstrin homology-like domain, family A, member 1). To test whether this gene is repressed directly by EWS/FLI1, we employed luciferase reporter assays, detailed promoter analysis using deletion mutants as well as point mutations. The corresponding results, together with chromatin immunoprecipitation assay data. provide evidence that PHLDA1 is a new EWS/FLI1 target gene. Since PHLDA1 has been suggested to play tumor suppressor role in melanoma and breast cancer, we would like to elucidate its functional relevance in Ewing's sarcoma. Hence, we established gain and loss of function systems and functional analysis of PHLDA1 is ongoing.

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Role of sonic hedgehog signaling in malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) tumorigenesis is associated with asbestos fibres in the pleural space causing a chronic tissue repair (reviewed in Stahel et al 2009). The presence of an 11-gene signature, correlated with a stem-cell-like expression profile, is associated with a poor prognosis in patients with MPM (Glinsky et al 2005). To address stem/progenitor cell signaling presence in MPM, we investigated the mRNA level of the sonic hedgehog (Shh) ligands Shh, Dhh and Ihh, Smo and Patch1 receptors, Gli-1 and Gli-2 transducers in normal pleura and MPM samples. Expression of Dhh and Ihh was observed in both MPM and normal pleura while Shh could be detected only in MPM samples. The expression of Gli-1, which is also a downstream target gene, was significantly higher in MPM tissue compared to normal pleura and nuclear Gli-1 staining was confirmed by immunohistochemistry. Primary MPM cell cultures were established in absence of serum and in 3% oxygen to prevent differentiation. In these culture conditions, the expression of Shh signalling pathway components was maintained. Conditioned medium from MPM ZL55SPT cells significantly activated a sonic hedgehog activity reporter system and the Smo antagonist cyclopamine, inhibited gli-1 expression, suggesting an autocrine signaling. In addition HhAntag, which is a more potent Smo antagonist compared to cyclopamine, inhibited cell growth and survivin expression in a dose-dependent manner. Survivin expression is a downstream target of the transcription factor YAP, which has been recently described as being positively regulated by Shh pathway (Fernandez et al 2009). Yap is expressed in MPM tumor tissue and cell cultures, and we observed that HhAntag treatment decreased YAP protein but not mRNA expression. Overexpression of Gli-1 partially inhibited HhAntag effect on survivin expression.

In addition, HhAntag treatment also increased the detection of truncated inactive Gli-1 forms, indicating that part of HhAntag effects might be due to regulation of Gli-1 protein. Finally, HhAntag administration to mice

bearing an MPM xenograft resulted in a significant decrease of MPM growth. Taken together, these data indicate that Shh signaling is necessary for MPM growth.

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Expression of base excision repair genes in response to differential tissue oxygenation involves multiple pathways and associates with cancer

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The base excision repair (BER) enzyme DNA polymerase (pol) λ is efficient in performing error-free translesion synthesis past the oxidative damage lesion 8-oxo-guanine (1). This lesion is, apart from depurination, the most abundant (103-104 modifications in each cell every day) and one of the most mutagenic in human cells, resulting in G:C->T:A transversion mutations that are frequently found in human tumor-relevant genes (2). Although, it has been occasionally reported that BER enzymes are overexpressed in tumors (3), neither control of their expression nor their role in tumorigenesis has not been clearly determined. Moreover, the extent to which DNA damage caused by continuous exposure to reactive oxygen species (ROS), or in contrast, oxygen deprivation (e.g. tumor hypoxia) affects expression of oxidative damage repair proteins also remains unknown. Initially, three transformed (MCF7, HeLa, U2OS) and one immortalized (293) human cell lines were tested for expression of the BER pols λ, β and the positive control pol ι in response to the hypoxia-mimicking drug dimethyloxalylglycine (DMOG). Treatment of the cells with DMOG resulted in up- or down- regulation of pol genes in transformed and immortalized cells, respectively. Subsequently, the same cell lines were harvested in an atmosphere containing 1% (hypoxia) or 21% (ambient air) O_2. The expression of pol genes was up-regulated for both types of cell lines in response to hypoxia. Similar findings were observed for primary lung fibroblasts, MRC5. At the same time, expression of pol λ in contrast to pol β and pol ι, was not affected in HIF1α (and HIF2α) deficient 293 cells suggesting a HIF-independent mechanism for its regulation. Further studies also revealed significant changes in expression and function of the two DNA glycosylases OGG1 and MUTYH, that, in addition to pol, contribute to elevated cell survival under hypoxia and re-oxygenation cycles. In parallel, animal tumor samples from dogs were analyzed and significant down-regulation of pol λ along with up-regulation of pol β and pol ι was detected in tumor tissues as compared to the reference tissue controls from the same dog. In summary, our current data suggest an involvement of different pathways in regulating the expression of BER genes in normal and transformed (tumor) human and animal tissues in response to oxygenation.

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Overexpression of CPT1C promotes migration and invation in cancer cell lines

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Background: All solid tumors, independent of their origin, contain areas of low oxygen concentration. Hypoxic cells are highly aggressive, metastatic and mostly resistant to therapeutic stimuli, mainly through the induction of genetic and epigenetic changes. Tissue invasion and metastasis mark the most lethal step of cancer disease and account for the majority of related deaths. Although very little is known about the molecular signaling pathways that trigger cancer metastasis, hypoxia is thought to activate signaling pathways that stimulate invasiveness of cancer cells.
We recently identified carnitine palmitoyltransferase 1C (CPT1C) as a p53-regulated brain-specific carnitine palmitoyltransferase (CPT) 1-family member that is up-regulated in response to hypoxia (manuscript submitted). This hypoxia phenotype, and the fact that p53 is a strong tumour suppressor, implicates a role of CPT1C in carcinogenesis.
Material and methods: Loss- as well as gain-of-function analysis of CPT1C was performed in various human tumour cell lines. To investigate the migration and invasion potential of these modified cancer cell lines, we used standardized scratch assays as well as Boyden chamber assays. CPT1C expression was examined in 54 pediatric brain tumors by real-time RTPCR, normalized to GAPDH and correlated to the hypoxia marker PGK.
Results: Here we report that the brain-specific CPT1C is a p53 target gene, induced by hypoxia and glucose deprivation in an AMPK-dependent manner. In order to investigate whether *in vivo* CPT1C expression correlates with tumor hypoxia, we examined 54 pediatric brain tumor samples. We found a significant correlation between PGK, the tumour hypoxia marker, and CPT1C mRNA expression ($p=0.013$). Strikingly, tumour cells engineered to constitutively express CPT1C showed increased proliferation, as well as increased fatty acid oxidation and ATP production, whereas cancer cells deficient in CPT1C function exhibited reduced proliferation, ATP production, sensitivity to hypoxia and glucose deprivation. Similarly, CPT1C-deficient murine embryonic stem cells showed altered fatty acid homeostasis and sensitivity to hypoxia and glucose limitation. Interestingly, all CPT1C gain-of-function cancer clones, independent of parental cell line and CPT1C expression level, showed a dramatic increase in migration and invasion potential in the Boyden chamber assays. Morphological analysis of these CPT1C gain-of-function clones revealed an increase of cytoskeletal structures if compared to the control clones. This relationship is further supported by immunoblots showing a significantly higher expression of tubulin in CPT1C gain-of-function clones compared to control clones, tubulin expression being an index of increased motility.
Conclusions: Our results indicate that tumour cells may protect themselves against hypoxic stress via CPT1C induction, perhaps by making fatty acids as an alternative fuel source, leading to a more aggressive phenotype in cancer cells. Tumor hypoxia is a major therapeutic concern because it reduces the effectiveness of radio- and chemotherapy and promotes metastasis. While therapeutic strategies that capitalize on chronic hypoxia sensitivity are available, no cellular targets have yet been characterized that induce cellular sensitivity to hypoxia. Understanding how CPT1C expression influences cancer cells might give new insight for the prediction of therapeutic efficiency and, furthermore, may lead to novel approaches for the therapy of otherwise treatment-resistant tumors.

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DNA Re-Replication: Molecular Features and Cellular Responses

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Eukaryotic cells initiate replication at specific chromosomal sites called origins of replication. To maintain genomic stability, cells restrict origin firing to only once per cell cycle. Deregulation of key replication factors, such as Cdt1 and Geminin, leads to re-replication, a particular type of replication stress triggered by re-firing of already initiated origins of replication. This event has been investigated in different model organisms and is predicted to lead to double strand breaks (DSBs), a source of genetic instability that contributes to tumorigenesis. However, the exact genotoxic process in mammalian cells and the consequences of re-replication at the DNA level, remain to be elucidated. Here we show that depletion of early mitotic inhibitor 1 (Emi1), a regulator of origin activity upstream of Cdt1, leads to massive DNA re-replication in U2OS cells as detected by flow cytometry. We analysed these re-replicating cells by immunofluorescence (IF) and flow cytometric analysis to follow, at single cell level, activation of the DNA damage response and

rate of DNA synthesis. We found that re-replication is rapidly associated with slower incorporation rates and subsequently leads to checkpoint activation, confirmed by Western blot analysis. Importantly, both IF and pulse field gel electrophoresis analysis showed no evidence of DNA breakage. Treatments of Emi1-depleted cells with checkpoint inhibitors further increased the extent of re-replication, apparently still in the absence of detectable DSBs. Thus, despite deregulated origin firing, re-replication is associated with an overall slowing of DNA synthesis and signals to the DNA damage checkpoints by DNA lesions other than DSBs, which in turn prevents further re-replication. A more detailed analysis of *in vivo* replication forks by electron microscopy and a quantitative investigation of origin activity by DNA combing in re-replicating cells are underway.

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Functional Role of ARTD1/PARP1 and ARTD2/PARP2 in Cell Cycle Progression

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The super family of diphtheria toxin-like ADP-ribosyltransferases (ARTDs) consists of 18 proteins of different structure and function (1). ADP-ribosyltransferase Diphtheria Toxin-like 1 (ARTD1 formerly called PARP1) is the most abundant and well-studied member. It contributes to several cellular functions such as cell death, chromatin stability and transcriptional regulation (2). ARTD2 (formerly called PARP2), another enzymatic active family member, can heterotetramerize with ARTD1, but its physiological role is still unclear. However, double knockout mice (ARTD1-/-; ARTD2-/-) are not viable (3). Revealing the functional contribution of ARTDs and their enzymatic activities during the cell cycle progression is of great importance. Hence, we aim to elucidate the role of ARTD1 and ARTD2 and its enzymatic activity during the cell cycle on a molecular level and to determine the functional relevance of the polymer formation. The urinary bladder cancer cell line (T24) serves as model with the particular property of being arrested in G0 phase upon reaching 100% confluency. Cells are released from the arrest in a synchronized manner after splitting, without reqiring the addition of other mitogens or other chemical agents to synchronize cells. After transfecting T24 cells with siARTD1, cells progressed more slowly through the cell cycle when compared to siMock treated cells. By using proliferation assays, we observed that delay occurs in late G1 phase and during G1/S-phase transition. Interestingly, cell cycle checkpoint controls (e.g., Chk1 phosphorylation) were not activated, indicating that the knockdown of ARTD1 per se does not lead to genotoxic stress. The progression of the cell cycle is tightly regulated by a network of different transcription factors and cyclin-dependent kinase complexes. No change in E2F-1 mRNA expression and protein levels could be detected upon siARTD1 treatment. CDK2 and p21 mRNA expression were also not affected. However, the cyclin E expression was completely abrogated in the absence of ARTD1. When T24 cells were transfected with siARTD2, cells accumulated in an early stage of the cell cycle (early G1). In contrast to the siARDT1 cells, cyclin E expression was not affected in siARTD2 cells. Surprisingly, the cdk inhibitor p27 mRNA expression and protein levels were found to be strongly increased in early G1 upon siARTD2 treatment.
In addition, we treated T24 cells with PARP inhibitors (e.g. ABT-888, Olaparib). By using flow cytometry analysis, a strong accumulation of inhibitor-treated cells in S phase was observed. This arrest did not correlate with the knockdown experiments of ARTD1 and ARDT2, indicating that the enzymatic activity may play a different role and even other ARTD family members may contribute. In summary, our results provide evidence for the importance of ARTD1 and ARTD2 and the synthesis of ADP-ribose for different cell cycle phases.

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Regulators of the oncogenic transcription factor PAX3-FKHR: Is PLK-1 a new therapeutic target in alveolar rhabdomyosarcoma?

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Oncogenic transcription factors are the second most frequently mutated class of proteins. They represent ideal therapeutic targets because oncogene addiction is common to a broad spectrum of tumors. However, thus far it has not been possible to target these oncogenic transcription factors as they usually do not respond to small-molecule inhibition. In order to develop a new strategy for targeting we use alveolar rhabdomyosarcoma (aRMS) as a model system which is characterized by the expression of the tumor-specific chimeric transcription factor PAX3-FKHR that is essential for survival of tumor cells. This transcription factor is post-translationally modified by phosphorylation which is required for efficient transcriptional activity. In addition it has been shown that treatment of aRMS cells by the broad spectrum kinase inhibitor PKC412 results in a strong antitumorigenic effect *in vitro* and *in vivo* (1).
Therefore we wanted to identify upstream protein kinases mediating PAX3-FKHR phosphorylation. We screened a siRNA library against the human kinome and a small compound kinase-inhibitor library. We used an endogenous cellular model, Rh4, which represents a PAX3-FKHR bearing aRMS with a transcription profile very similar to tumor biopsies. As a read-out system we simultaneously assessed cell viability together with a well established and highly sensitive luciferase reporter assay based on the AP2beta target gene promoter to monitor fusion protein activity (2, 3). The siRNA screen against the human kinome identified PLK-1 and 46 other candidate kinases to have an inhibitory effect on the activity of PAX3-FKHR upon their silencing. In a further subscreen PLK-1 knockdown had the greatest negative effect on cell viability in two different translocation-positive alveolar rhabdomyosarcoma cell lines. Also screening of the small compound kinase-inhibitor library identified BI-2536, a drug targeting PLK-1, as the most effective compound. Moreover preliminary data of drug treatment in vivo using BI-2536 resulted in a regression of tumor volume in a NOD/ScidIl2rg-/- xenograft mouse model. These data suggest that PLK-1 warrants further investigations as a novel drug target for the treatment of aRMS.

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CD133 positive Rhabdomyosarcoma stem-like cell population is enriched in Rhabdospheres

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Cancer stem cells (CSCs) have been identified in a number of solid tumors, but not yet in Rhabdomyosarcoma (RMS), the most frequently occurring soft tissue tumor in childhood. Hence, the aim of this study was to identify and characterize a CSC population in RMS using a functional approach.
We found that RMS cell lines can form rhabdospheres in stem cell medium containing defined growth factors over several passages. Using an orthotopic xenograft model, we demonstrate that a 100 fold less sphere cells result in faster tumor growth compared to the adherent population suggesting that CSCs were enriched in the sphere population. Furthermore, stem cell genes such as oct4, nanog, c-myc, pax3 and sox2 are significantly upregulated in rhabdospheres which can be differentiated into multiple lineages such as adipocytes, myocytes and neuronal cells.
Surprisingly, gene expression profiles indicate that rhabdospheres show more similarities with neuronal than with hematopoietic or mesenchymal stem cells. Analysis of these profiles identified the known CSC marker CD133

as one of the genes upregulated in rhabdospheres, both on RNA and protein levels. CD133+ sorted cells were subsequently shown to be more tumorigenic and more resistant to commonly used chemotherapeutics. Using a tissue microarray (TMA) of eRMS patients, we found that high expression of CD133 correlates with poor overall survival. Hence, CD133 could be a prog-nostic marker for eRMS.
These experiments indicate that a CD133+ CSC population can be enriched from RMS which might help to develop novel targeted therapies against this pediatric tumor.

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Amplification of the centromeric region of chromosome 17 is a rare event, comprising further amplified genes on the long arm of chromosome 17 and presenting a potential diagnostic pitfall in Her2 testing

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Aim: Correct evaluation of centromere (CEP17) and Her2 signals by Her2 fluorescence in situ hybridization (FISH) testing is essential for therapy purposes in breast cancer. Aberrant patterns (as clusters) on CEP17 can be misleading in calculation of Her2/CEP17 ratio and can result in false positive or false negative Her2 status, which is of enormous consequence for the patient. Anti-Her2 therapies are expensive treatments with considerable side effects. We retrospectively analyzed 14 breast cancer cases with cluster-forming chromosomal gains on CEP17/Her2.
Methods: We compared evaluation of Her2/CEP17 ratios between three institutions. Afterwards we tested CEP17 amplicon with two different probes (17p11.1 - q11.1 and 17p11.2 - p12). Furthermore we analyzed potentially involved regions on chromosome 17 (p53, RARA, Top2) by FISH. Her2 gene was tested with FISH/silver-ISH(SISH). The Her2 protein was determined immunhistochemically (IHC).
Results: Overall agreement on Her2/CEP17 ratio was 64% (9/14 cases). Discrepant ratios varied from 1.1 to 14.3. 17p11.2 - p12 was eusomic in 79% (11/14), 17p11.1 - q11.1 in none (0/14). RARA gene was amplified in 11/14 cases (79%), Top2 gene in 3/14 cases (21%). P53 was eusomic in 14/14 cases (100%). Her2 gene was amplified with FISH/SISH in 14/14 cases (100%). 9/14 tumors were 3+ IHC positive (64%).
Conclusion: Large Her2/CEP17 amplicons are problematic and can result in inaccurate Her2-FISH/SISH status. Careful assessment of adjacent genes and additional Her2-IHC are of critical importance in such cases.

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Discovery and validation of new stratification markers in childhood acute lymphoblastic leukemia

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Relapse of acute lymphoblastic leukemia (ALL) is a leading cause of cancer-related childhood mortality. A group of patients with a very high risk of relapse (VHR-ALL) can be identified by analysis of treatment response based on the persistence of significant levels of minimal residual disease (MRD) after almost 6 months of treatment[1]. These patients would qualify for new experimental approaches as part of their initial therapy. Despite impressive advances in the molecular characterization of ALL, prognostic markers are

not available to identify this subgroup at diagnosis.
Here we aimed for a description of leukemia cell surface protein landscape which would in addition allow us to compare patients from two clinically relevant risk groups, namely VHR-ALL and patients with a good clinical outcome (standard risk, SR-ALL).
Given a limited amount of material available for research, we used a mouse xenotransplantation model to expand scarce bone marrow samples from ALL patients for proteomic studies. The cell surface glycoproteome of expanded ALL cells from 8 VHR-ALL and 11 SR-ALL patients was analyzed using cell surface capturing (CSC) technology[2]. This mass spectrometry analysis identified 978 proteins on the cell surface of ALL. Next to many unanticipated proteins found on the surface of ALL cells, we were also able to recapitulate the immunophenotype described at diagnosis with currently used leukemia markers such as CD45, CD34, CD38, CD22, CD19, and CD10. Furthermore, eight candidate makers for ALL risk stratification were selected based on predominant protein identifications in one risk group or large protein abundance differences between the two risk groups. Flow cytometry analyses of these candidate markers identified vanin 2 (VNN2) as the most promising candidate risk stratification marker for a subgroup of VHR-ALL patients. Subsequent validation of this result in an independent cohort of 600 patients by quantitative PCR confirmed elevated expression of VNN2 in a subgroup of VHR-ALL compared to SR-ALL cases. Gene expression profiling of this subgroup in a second independent dataset revealed a distinct gene expression signature for VNN2 high ALL patients compared to low VNN2 expressors. Among the genes overexpressed in VNN2 high group we identified several cytokines, cytokine and chemokine receptors and genes associated with innate immunity and inflammation. Indeed, gene set enrichment analysis identified genes related to response to inflammation and to external stimuli to be most highly enriched in the VNN2 high signature. These findings provide the basis to evaluate the functional role of VNN2 in high risk disease.
Taken together, our data provide an unprecedented view at the cell surface landscape of the most refractory leukemia cases and identify cell surface proteins that could be amenable for diagnostic use and/or therapeutic intervention in this deadly disease.

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Modulation of Matrix Metalloproteinase 1 (MMP-1) expression in Human Osteosarcoma cells directly affects intratibial tumor formation and lung metastases in mice

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Osteosarcoma is the most frequent primary malignant tumor of bone, predominantly affecting children and young adults. Patients with metastatic disease at diagnosis have a poor prognosis, reflected by 5 year survival rates of only 20-30%. Metastatic progression is a complex process in which tumor cells invade distant target organs. Several steps during this process, like the degradation of extracellular matrix, require extracellular proteolytic enzymes. Overexpression of MMP-1, a member of the matrix metalloproteinase family, has been associated with poor prognosis in a variety of human cancers.
LacZ-tagged HOS (HOS-LacZ) cells stably overexpressing MMP-1, 143B (143B-LacZ) cells downregulated in MMP-1 expression by siRNA and control cell lines were generated by retroviral infection. These cell lines were used in different *in vitro* assays (cell adhesion assay and soft agar colony formation assay) and an orthotopic *in vivo* tumor model to investigate the functional relevance of MMP-1 in osteosarcoma metastasis.
MMP-1 was found upregulated in the highly metastatic 143B osteosarcoma cells in comparison to its parental, non-metastatic HOS cells. The biological relevance of this finding was further investigated *in vitro* and *in vivo*. Overexpression of MMP-1 in HOS-LacZ cells enhanced adhesion to collagen type I more than 2-fold compared to control cells and facilitated anchorage-independent growth, reflected by fast-growing cell colonies in soft agar. Conversely, siRNA-mediated downregulation of MMP-1 expression in 143B-LacZ cells inhibited the adhesion to collagen type I and reduced the number of fast-growing cell colonies in soft agar 3-fold. These findings in vitro suggested that robust expression of MMP-1 in 143B-LacZ cells and in stably MMP-1 infected HOS-LacZ cells may have a significant impact on the metastatic activity of these cell lines *in vivo*. This was indeed confirmed in SCID mice upon intratibial injection of MMP-1 expression modified HOS-LacZ and 143B-LacZ cells, respectively, and of the corresponding control cells. MMP-1 overexpressing HOS-LacZ cells, unlike the control cells, formed intratibial, osteolytic primary tumors and numerous micrometastases in the lung. Conversely, 143B-LacZ cells with siRNA-downregulated MMP-1 expression

formed smaller intratibial primary tumors and a significantly lower number of lung macrometastases than the control 143B-LacZ cells. In conclusion, MMP-1 is a key modulator of intratibial primary tumor growth and of lung metastases of human 143B and its parental HOS osteosarcoma cells in mice.

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Intralesional transcriptional adaption in advanced cutaneous T-cell lymphoma (CTCL) caused by vorinostat/bexarotene therapy

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Objective: To evaluated intralesional molecular adaptation during the phase I study of vorinostat/bexarotene combination therapy in patients with advanced CTCL (Clin. Trials Nr. NCT00127101).
Design: Transcriptional profiling was performed on pre- and on-treatment (day 14 into 1st cycle) skin biopsies from 15 patients. In several cases both, tumor and plaque lesions were available for the analysis. Human Exon 1.0 ST microarrays by Affymetrix were used and transcriptional modifications were identified by Partek® and R-Bioconductor, enrichment analysis (EA) was performed using Genego Metacore®.
Results: Analysis of expression differences and alternative splicing showed that the magnitude of changes was relatively small. Interindividual patient's variability was identified as the major factor influencing the molecular response. Plaque and tumor biopsies did not show any differences. EA of differentially expressed genes revealed that progressive patients upregulate NOTCH and ER stress-responsive cascades and downregulate JAK-STAT signaling. In benefiting patients hormonal response network was the most represented with the emphasis on cell survival and inflammatory response (PPRG, RXR, RXRB, MIF, ELK1, HDAC1, SOX5). Angiogenesis regulation through IL-8 and apoptosis were downregulated. Evaluation of alternative splicing after 14 days of the therapy showed that genes involved in cytoskeleton and matrix remodeling (MMP2, COL1A2, E-cadherin), cell proliferation (JAK2, ERK1/2, STAT5), and other processes tend to be modified.
Conclusions: Vorinostat/bexarotene treatment caused consistent adaptions in the intralesional transcriptome. Progressive patients seem to escape through NOTCH signaling. Responsive patients are characterized by apoptosis induction upon treatment. Results may help to initiate the development of personalized therapeutic strategies targeting responsive CTCL patients.

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Hypoxia is a driver in melanoma phenotype switching

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Our group reported a model for melanoma progression which involves melanoma cell switching between phenotypes of invasion and proliferation. In this study we examine the possible role of hypoxia as one of the microenvironmental influences driving metastatic progression in that it may promote the shift from a proliferative to an invasive phenotype. In initial experiments, microarray analyses were performed on proliferative phenotype melanoma cells exposed to hypoxic conditions *in vitro*. These experiments showed an up-regulation of invasive phenotype-specific genes as well as a down-regulation of proliferative phenotype-specific genes (including important melanocytic markers). *In vitro* invasion assays were performed to show that a hypoxic environment increased the invasiveness of proliferative melanoma cell cultures. Importantly, invasiveness was retained after these cells were returned to normoxia for 48 hours. Furthermore, extended periods of hypoxia increased invasiveness in proliferative phenotype cells in a dose-dependent fashion. In contrast, invasive phenotype melanoma cells showed no increase in invasive potential upon exposure to hypoxia. Additionally, we stained Clark's level IV primary human cutaneous melanoma biopsies for key markers concerning melanocytic function, hypoxia, proliferation and vascularisation. Hypoxic regions correlate with a loss of melanocytic marker expression, supporting our *in vitro* data. These results suggest that exposure to a hypoxic microenvironment leads to the reprogramming of proliferative phenotype melanoma cells, down-regulating melanocytic marker expression and increasing their invasive potential. Separately, we also pursued the development of a novel tool for predicting melanoma cell phenotype based

on gene expression profiling. Using microarray data we created phenotype-specific meta-standards based on specific genes as expressed across 192 melanoma cell cultures. Based on this we developed a protocol to mathematically assess the similarity of the gene expression data from individual samples to proliferative and invasive phenotype meta-standards. This tool allows us to make predictions concerning both phenotype and phenotype switching in different experimental contexts.

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Dendritic cell-based therapy for experimental glioblastoma

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Glioblastoma (GBM) is the most common malignant brain tumor in adults and despite multimodal treatment, the prognosis remains poor (1). Furthermore, resistance to treatment and tumor recurrence are thought to be due to a small subset of tumor cells termed cancer stem cells (CSCs). This population of cells has been shown to be highly proliferative, self-renewing and multipotent, with the ability for tumor formation when injected intracranially into mice. To characterize the CSCs and their tumor forming ability *in vivo* we used the naturally occurring spontaneous mouse astrocytoma (SMA)-560 cell line, derived from VM/Dk mice, to implant tumor adherent (bulk) cells or non-adherent (sphere) cells, enriched in CSCs. Another unique feature of GBM that prevents an effective anti-tumor response and promotes tumor progression is the ability to escape immune surveillance. This is achieved by the creation of an immunosuppressive microenvironment through the release of immunosuppressive TGF-beta 538;2, IL-10 and prostaglandins. This has led to treatment strategies focusing in the direction of immunotherapy. One such immunotherapeutic strategy is the expression of soluble CD70 (sCD70), a co-stimulatory molecule required for T cell activation. Mice bearing sCD70 expressing gliomas have been shown to promote antiglioma immune responses through a CD8-mediated mechanism resulting in prolonged survival (2) and antitumor T cell memory (3). In our studies we have established SMA-560 bulk and sphere cells that either stably express a sCD70 transgene or a control vector. Our preliminary data suggest a survival advantage and an increased antiglioma response in mice bearing gliomas induced by both sCD70 expressing bulk and sphere cells. Alongside exploiting the immune stimulatory effects of sCD70, we are also attempting to develop a dendritic cell-based vaccination strategy that specifically targets CSCs.

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Spontaneous liver cancer in mice with hepatocyte-specific deletion of the anti-apoptotic protein Mcl-1: An *in vivo* model for apoptosis-driven cancer

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Background and aims: Apoptosis is not only important for embryonic development but also plays a decisive role in tissue homeostasis. By removing unwanted or dangerous cells (e.g. those with oncogenic alterations), apoptosis is considered a tumor-preventing mechanism. Decreased sensitivity towards apoptosis is associated with hepatocarcinogenesis and non-responsiveness to therapy-induced cell death. Elevated sensitivity of hepatocytes to cell death signals may result in chronic liver injury. Many human chronic liver diseases (e.g. viral hepatitis) are characterized by increased apoptosis of liver cells. We have developed a murine model with a hepatocyte-specific knockout of the Myeloid cell leukemia-1 (Mcl-1) gene in order to recapitulate the constant loss of liver cells observed in various human liver diseases (1).
Results: Deletion of the anti-apoptotic Bcl-2 protein Mcl-1 in hepatocytes caused spontaneous hepatocyte apoptosis and severe liver damage. This

was paralleled by a (compensatory) increased proliferation of hepatocytes (1) leading to spontaneous Hepatocellular carcinoma (HCC) formation (2). At the age of eight months > 50% of the Mcl-1 deficient animals had developed liver tumors. Tumors ranged from small dysplastic nodules to frank HCC. This was proven by histology, expression of established HCC markers and chromosomal aberrations. HCC revealed a remarkably heterogenous morphological spectrum and immunophenotypes. Comparative genomic hybridization analyses of different tumors showed several deletions and amplifications, but no mutual pattern was observed. Expression analyses of 16 particular genes suggested that HCC could be divided into two groups: one low proliferating and highly differentiated, and the other highly proliferating and highly differentiated.
Conclusions: Our observation that constant loss of hepatocytes, caused by the lack of Mcl-1, leads to compensatory proliferation and thereby triggers hepatocarcinogenesis adds another level of complexity to the interplay between apoptosis and carcinogenesis. Not only resistance against apoptosis is a hallmark of cancer, but also an increased number of apoptotic cells can be cancerogenic. Our observation has profound implications for the understanding of hepatocarcinogenesis(3).

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Gene Regulation by Endogenous Retroviruses Can Cause Cancer

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Human endogenous retroviruses (HERVs) constitute approximately 8% of the human genome sequence. By comparison with their high abundance, little is known about the overall impact of HERVs on gene regulation, or any relevant underlying mechanisms. Recently, the generation of cis-acting antisense transcripts has been proposed as a common gene regulation mechanism exhibited by HERVs (1). In this context, the long terminal repeats (LTRs) flanking a HERV provirus in particular have the ability to act as promoters. We chose the Death-associated protein 3 (DAP3) gene as a model for regulation by an endogenous retrovirus; this being one of the first experimentally verified examples. DAP3 is described as a positive mediator of apoptosis (2). We demonstrated that a member of the HERV-K(C4) family endogenous retrovirus that is integrated in the first intron of the DAP3 gene negatively regulates expression of the encompassing gene. It does so by providing a cis-acting antisense transcript originating
from the 5' LTR of the provirus. We verified bidirectional promoter activity of this LTR in vitro that could be suppressed by interferon-gamma and found evidence for promoter activity in vivo. In a top down approach to confirm its presence, we knocked down the putative antisense transcript in HeLa cervix carcinoma cells with specific antisense oligonucleotides. As expected, DAP3 expression was induced and cells underwent apoptosis, consistent with DAP3 being a pro-apoptotic protein. Additionally, we propose a model in which the HERV-K(C4) provirus in the DAP3 gene is causally implicated in human tumorigenesis. One of the hallmarks of virtually all tumors is the ability to evade apoptosis. Since DAP3 is a pro-apoptotic gene, its down-regulation might contribute to apoptosis resistance of malignant cells. In this context, DAP3 has been shown to be deregulated in numerous human cancers including glioblastoma, neuroblastoma and leukemia. A further feature of many human tumors is the up-regulation of HERV-K transcriptional activity (3). Consequently we propose the identified HERV-K(C4) primed DAP3-specific antisense transcript to be particularly abundant in tumor cells, down-regulating DAP3 expression thus rendering them resistant to apoptosis. While exogenous, infectious human retroviruses such as the Human Immunodeficiency Virus-1 or the Human T-Lymphotropic Virus-1

are well-understood as direct or indirect tumor-inducing agents, a causal role of HERVs in human tumorigenesis could not have been verified yet, although there is numerous evidence. The HERV-K(C4) provirus in the DAP3 gene might be the first example of an endogenous retrovirus actively taking part in human tumorigenesis by promoting resistance to apoptosis. Further work is necessary to prove this hypothesis.

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Role of protein covertase furin in pediatric soft tissue sarcomas

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Furin, the best characterized member of the protein convertases (PCs) family, is associated with tumors because of its role in the processing and activation of proteins involved in cellular transformation, tumor progression and angiogenesis, such as MMPs and VEGFs. In fact, furin upregulation has been observed in several tumors. Rhabdomyosarcoma (RMS), is the most common soft tissue sarcoma in children. We found that chemotherapeutic drugs can be specifically delivered to RMS using targeting peptides binding to furin. Moreover, we found a strong correlation between PCs activity and RMS growth: by inhibiting PCs in embryonal (eRMS) and alveolar (aRMS) rhabdomyosarcoma cell lines we obtained an impressive difference in the *in vivo* tumor growth, even if the cells show the same growth *in vitro*. In the light of these facts, we will perform *in vivo* experiments with RMS cells with silenced furin of PC7, the two PCs expressed in RMS. We hypothesise that in the tumor microenvironment the action of furin or PC7 is able to accelerate the growth by increasing the vascularization of the tumors. Therefore, tumors will be collect at different stages and cell proliferation, hypoxia and angiogenesis will be compared. Confirming the relation between furin activity and RMS growth will validate furion as therapeutic target, contribute to a better understanding of RMS progression and may be useful to devise better therapies.

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Bone morphogenetic protein-7 is a MYC target with prosurvival functions in childhood medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor in children (1). It is known that overexpression and/or amplification of the MYC oncogene is associated with poor clinical outcome, but the molecular mechanisms and the MYC downstream effectors in MB remain still elusive. Besides contributing to elucidate how progression of MB takes place, most importantly, the identification of novel MYC target genes will suggest novel candidates for targeted therapy in MB. A group of 209 MYC-responsive genes was obtained from a cDNA microarray analysis of a MB-derived cell line, following MYC overexpression (2) and silencing. Among the MYC-responsive genes, we identified members of the bone morphogenetic protein (BMP) signaling pathway, which plays a crucial role during the development of the cerebellum (3). In particular, the gene BMP7 was identified as a direct target of MYC. A positive correlation between MYC and BMP7 expression was documented by analyzing two distinct sets of primary MB samples. Functional studies *in vitro* using a small-molecule inhibitor of the BMP/SMAD signaling pathway reproduced the effect of the siRNA-mediated silencing of BMP7. Both approaches led to a block of proliferation in a panel of MB cells and to inhibition of SMAD phosphorylation. Altogether, our findings indicate

that high MYC levels drive BMP7 overexpression, promoting cell survival in MB cells. This observation suggests the potential relevance of targeting the BMP/SMAD pathway as a novel therapeutic approach for the treatment of childhood MB.

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CD133+ or CD44+CD166+ cells from human colorectal cancer cell lines do not display cancer stem-cell features nor increased drug resistance

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Background: Tumour initiation and maintenance is caused by rare tumour cell subsets, defined as "cancer stem cells" (CSCs), endowed with self-renewal and differentiation capacity. CSCs have a number of properties permitting them to survive conventional cancer chemotherapy and radiotherapy. The development of alternative therapeutic approaches specifically targeting CSCs is urgently needed. Primary screening of novel anti-cancer compounds is conventionally conducted on established tumour cell lines, which are easy to propagate *in vitro* and amenable to high throughput studies. However, whether they do actually comprise CSC populations resembling those of primary tumours remains controversial. We performed phenotypic and functional characterization of putative CSC populations in established cell lines of human colorectal carcinoma (CRC) and evaluated their suitability for predicting efficacy of anti-cancer therapies.
Material and Methods: A panel of 10 established human CRC cell lines was studied. Expression of putative CSC markers, including CD133 or CD44/CD166 molecules, was evaluated by flow cytometry. CD133+ or CD44+ CD166+ cells were sorted from individual cell lines by flow cytometry and evaluated for CSC properties in comparison to their negative counterparts or to the parental cell line. Spheroid formation ability, clonogenicity, stemness-related gene expression, aldheyde-dehydrogenase-1 activity, side population phenotype, *in vitro* invasiveness, chemo-resistance and tumorigenicity upon injection in NOD/SCID mice were assessed.
Results: None of the putative CSC phenotypes analyzed was found to be significantly associated with functional features of CSC. Importantly, neither CD133+ nor CD44+ CD166+ cells showed significantly increased resistance to chemotherapeutic drugs currently in use for CRC treatment, as compared to their negative counterparts.
Conclusions: On established CRC cell lines, the expression of putative CSC markers does not correlate with CSC functional features. Our findings question the adequacy of established CRC cell lines for screening of CSC-specific therapies and underline the urgency to develop novel platforms for anti-cancer drug discovery.

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VHL missense mutations exert diverse effects on HIFα degradation and imply different predictive potential in sporadic clear cell renal cell carcinoma

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Mutations of the von Hippel-Lindau gene (VHL) are observed in about 70% of sporadic clear cell renal cell carcinomas. Approximately half of the mutations are nonsense and frameshift mutations which most probably abrogate the function of the VHL protein (pVHL). The consequences of missense mutations for pVHL's integrity are largely unknown. To address this question we sequenced VHL in 256 sporadic ccRCC specimens. VHL mutations were identified in 181 tumors (71%), 63 (35%) of which had missense mutations. We first characterized the VHL missense mutations by determining their locations and destabilizing effects *in silico*. The majority of destabilizing missense mutations were located in exon 1 in the core of pVHL, whereas all exon 3 mutations affected the interaction domains of elongin B/C on the protein surface. Their impact on pVHL's functionality was further investigated in vitro by stably reintroducing VHL missense mutations into a VHL null cell line and by monitoring the GFP signal after the transfection of a HIFα-GFP expression vector. pVHL's functionality ranged from no effect (Ser68Thr) to complete

HIF stabilization (Leu101Pro). Interestingly, Asn78Ser, Asp121Tyr, and Val-130Phe selectively influenced HIF1α and HIF2α degradation. By combining the results from our *in silico* and *in vitro* analyses, we obtained three different groups of missense mutations which.lead to a severe destabilization of pVHL (group A); had no destabilizing effects on pVHL but affected the interaction with HIFα, elongin B, or elongin C (group B); or had functionalities comparable to the wild-type protein. We conclude that dissecting the impact of specific missense mutations on pVHL's functionality may help to better predict the response of ccRCC patients to HIF targeted therapies.

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Mesenchymal stromal cells enhance the malignant potential of human colorectal cancer cells by inducing epithelial-mesenchymal transition (EMT)-related phenomena.

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Background: Mesenchymal stromal cells (MSCs) are recruited to primary and metastatic sites of several tumor types, including colorectal cancer (CRC), and might contribute to tumor progression. The actual role played by MSCs and the mechanisms underlying MSC-tumor interactions remain to be clarified. We investigated the effects of human bone-marrow-derived MSCs (BM-MSCs) and tumor-associated stromal cells (TASCs) on CRC, *in vitro* and *in vivo.*
Material and methods: Human established CRC cell lines were cultured in the presence or absence of BM-MSCs or TASCs, in direct contact or in transwell plates. After a five day culture, tumor cell proliferation was assessed by differential cell counts, surface molecule expression was analyzed by flow cytometry, and production of soluble factors in culture supernatants was measured by Raybio antibody array and ELISA. Tumor cells, sorted upon co-culture by flow cytometry, were evaluated for the expression of EMT-related genes by quantitative PCR and for *in vitro* invasiveness, by chemoinvasion assay. Furthermore, their tumorigenicity was assessed upon injection in NOD/SCID mice and developing tumors were analyzed by immunofluorescence.
Results: Stromal cells significantly increased tumor cell proliferation and decreased CD44 expression, independently of cell-to-cell contact. Analysis of co-culture supernatants revealed higher amounts of IL-6, MCP-1, RANTES and Angiogenin, in comparison to supernatants derived from single cultures. Moreover increased expression of several EMT-related genes, including SNAIL1, SNAIL2, TWIST, ZEB1 and N-Cadherin, was detected on CRC cells sorted upon co-culture as compared with controls. Importantly, CRC cells co-cultured with stromal cells showed higher invasive behaviour in vitro, than CRC cells cultured alone. No significant changes were observed in tumorigenicity. However, tumors originated from tumor cells co-cultured with MSCs showed a higher tumor size and vessel density as compared to controls.
Conclusions: Stromal cells reduce adhesiveness, induce expression of EMT-related genes and increase proliferation, invasiveness and angiogenic potential of CRC cells. These effects might contribute to CRC progression and spreading.

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High resolution divisional tracking reveals cycling dynamics of hematopoietic stem cells in steady state and upon hematopoietic challenge

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High-throughput and lifelong blood production is maintained by a small fraction of hematopoietic stem cells (HSCs). Steady-state HSC division kinetics have been evaluated by *in vivo* labeling assays with 5-bromo-2-deoxyuridine (Kiel et al., 2007), biotin (Nygren and Bryder, 2008) and histon 2B-green fluorescent protein transgenic mouse models (Trumpp et al., 2010). While the former studies showed that all HSCs equally divide and likely contribute to hematopoiesis (maintenance model), the latter suggested that HSCs are divided into two pools, cycling HSCs with limited self-renewal and likely high contribution to blood formation and quiescent HSCs with very few turnovers and rare hematopoietic contribution during lifetime (succession model). However, the labeling techniques previously used were unable to track single cell divisions and might have direct influence on cycling activity of HSCs. Thus, the HSC division rate and steady-state hematopoietic contribution, the relationship between divisional history and repopulating ability, and the impact of naturally-occurring hematopoietic challenges on HSC self-renewal and differentiation remain to be determined. To address these directly, we established high resolution non-invasive *in vivo* HSC divisional tracking with 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester (CFSE). We here show that i.v. transfer of CFSE-labeled HSCs into nonirradiated recipient mice allows to evaluate steady-state HSC cycling dynamics as CFSE is equally distributed to daughter cells upon cellular division, and revealed 0- and 1-7x divided LKS in recipient bone marrow over at least 20 weeks. Single cell and limiting dilution transplantation showed that both 0- and ≥5x divided LKS at ≥7 weeks after chase multilineage repopulated recipients and showed fluctuating lifelong reconstitution. Mathematical modeling revealed no evidence for a dichotomy of biologically defined HSC pools. Steady-state serial transplantation with fast-cycling cells revealed that they can slow down divisional rate over time, indicating dynamically changing cycling activity of HSC. We next tested the effects of lipopolysaccharide (LPS) injections on HSC proliferation. Analysis revealed that 2-4 time and ≥5x divided LKS from LPS-treated mice reconstituted multilineage blood cells whereas neither fraction from control mice engrafted. This data clearly indicate that HSCs are activated from quiescence upon LPS challenge, and provide in vivo evidence that naturally-occurring hemato-immunological challenges as gram-negative bacterial infection induces HSC proliferation and self-renewal. Our data suggest a novel "dynamic repetition" model in which cycling HSCs produce differentiated offspring for a while and subsequently enter a resting phase while dormant HSC get activated into cycle and follow the same fate as cycling ones, likely leading to homogenous turnover of the entire HSC pool at end of life. These findings suggest an evolutionally developed system to ensure equal distribution of work-load, demand-adopted recruitment of stem cells, and reduction of risk to acquire genetic alterations or fatal damage which might hold true for other somatic stem cell systems as well. Furthermore, the CFSE-tracking model established here now allows to test the role of intrinsic versus environmental cues on cycling dynamics of HSCs as well as leukemia-initiating cells (LICs) with multiple genetic and different species background, and might help in the development of specific and efficient therapies to target LICs.

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Leukemia initiating cells are frequent and detected in distinct clonal subsets in de novo resistant acute lymphoblastic leukemia

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For children with precursor B acute lymphoblastic leukemia (ALL) who relapse on current intensive chemotherapy regimens, second line therapy is challenging (1). Xenotransplantation of primary leukemia cells constitutes an attractive strategy to model disease for translational research, but the consequences of selective pressure on leukemia cells need to be clarified. Here we evaluate the phenotypic and genetic stability of primograft samples established by orthotopic xenotransplantation of leukemic cells from a group of patients with very high-risk precursor B-cell ALL (VHR-ALL), characterized by the persistence of minimal residual disease after intensive induction and consolidation therapy (2). Phenotypic properties remained unchanged after up to 5 passages in NOD/scidIL2Rgnull mice, with concordant immunophenotypes in 90% of measurements of 10 leukemia-associated markers and stable in vitro drug response profiles. In 4/6 VHR-ALL cases, 100 unsorted primografted ALL cells were sufficient to reconstitute the leukemia. Most of up to 7 recurrent copy number abnormalities (CNA) detected in diagnostic samples were maintained in primografts. Changes in CNAs were few with 0-4 new or lost deletions and/or amplifications per case and mostly deletions occurred during the first xenotransplantation (3). Most frequently aberrations on the p-arm of chromosome 9 (including CDKN2A/B, MTAP, PAX5) were detected. At the single cell level, the pattern of deletions in the CDKN2A locus revealed distinct subsets of ALL cells, which were reproducibly tracked in primografts by FISH analysis. Clonal IgH and TCR gene rearrangements were observed by PCR and most of them were stably detectable in patient and primografted material. These results demonstrate the existence of clonally closely related but distinct subsets of leukemia initiating cells in VHR-ALL, which has important implications for the use of this model.

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miR-21 Suppression Impedes Medulloblastoma Cell Migration

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Medulloblastoma (MB), the most common malignant brain tumour in children, is often found to be disseminated throughout the CNS by the time of diagnosis. Current therapy has not reduced the high mortality associated with tumour metastasis and little is known about the molecular mechanisms that promote cancer cell migration in this tumour. Aberrant expression of miR-21 was recently found to be causatively linked to metastasis in diverse human neoplasms including brain tumours however its function in MB is still unknown. In this study we investigated the expression level and the role of miR-21 in MB cell migration. miR-21 was found to be upregulated in all MB patient's samples and cell lines tested, compared to non neoplastic fetal cerebellum. Our observed inverse correlation between miR-21 and the metastasis suppressor PDCD4-protein, together with the finding that miR-21 repression increased the release of PDCD4 protein, suggests a potential regulation of PDCD4 by miR-21 in MB cells. In addition anti-miR-21 decreased the expression of the tumour cell invasion mediators MAP4K1 and JNK, which are negatively regulated by PDCD4, and decreased the expression of integrin protein, that is known to be essential for MB leptomeningeal dissemination. Moreover miR-21 knockdown in MB cells increased the E-cadherin and

TIMP proteins that are positively regulated by PDCD4 and known as negative modulators of cancer cell migration. Finally and importantly, suppression of miR-21 decreased the motility of MB cells and reduced their migration across basement membranes *in vitro*. Together this compelling data proposes miR-21 as a novel mechanism impacting MB cell dissemination and raises the possibility that anti–microRNA-21 may have potential therapeutic value in MB patients.

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New functional screening approaches based on automated microscopy for the development of new antileukemic strategies

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Protective signals from the host microenvironment are required for even the most aggressive form of childhood acute lymphoblastic leukemia (ALL), supporting the idea of a protective niche for resistant clones in the bone marrow. Targeting mechanisms in the bone marrow microenvironment that confer ALL survival could lead to new antileukemic strategies for relapse patients and patients resistant to current chemotherapeutic drugs. In vitro, co-culture of primary ALL cells on human mesenchymal bone marrow derived stromal cells (MSC) provides survival cues enabling long term cultures. In contrast, primary ALL cells rapidly undergo cell death when cultured without stromal support [1]. Interference with such pro-survival mechanisms represents an interesting strategy for therapy. Therefore we have developed an automated microscopy-based functional screening approach to define signals that originate from MSCs to mediate survival of ALL cells. Primary patient material from very high risk ALL patients with poor in vivo response to treatment was amplified in immunodeficient NOD/LtSz-scid IL2Rgammanull mice in our laboratory [2]. This model provides sufficient amounts of primary ALL cells from refractory ALL patients to perform for the first time functional screening studies with primary leukemia cells. The viability of ALL cells co-cultured with MSC cells is assessed by automated microscopy-based analysis, in collaboration with the RISC-LMC center at the ETH Zurich. Unfixed ALL cells co-cultured on MSCs in a 384 well-plate format are stained with a fluorescent nuclear dye and the plates are imaged using an automated microscope. Use of this stain enables discrimination of MSC nuclei from ALL cell nuclei due to the smaller size and brighter staining pattern of the latter. The living ALL cells are identified and quantified from the dual layer composed of ALL and MSC cells by a software called advanced cell classifier which was developed at the ETH by Peter Horvath. The advanced cell classifier is a machine learning program able to learn to differentiate between cells upon characteristics of the nuclear staining by implementing given information in a defined algorithm.

The number of living ALL cells assessed by the advanced cell classifier software from pictures taken by the automated microscope showed a high correlation to the living cell numbers counted by flow cytometry detection of 7-AAD-negative living cells. This validates our procedure as an accurate high-throughput screening method. We performed a siRNA screen down-regulating candidate proteins at the stromal level on the basis of results generated by proteomic and microarray analyses and are performing compound screens in order to identify antileukemic agents. These two strategies will elucidate which cellular pathways are involved in drug resistances and lead to the discovery of new potential antileukemic agents for more effective combinatorial treatments for refractory patients.

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Global analysis of genotoxic stress signaling by reverse phase protein microarray

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Genotoxic stress is a life-threatening event for organisms as it alters the content and organization of the genetic material. DNA lesions are detected by sensor proteins, which recognize either the lesions themselves or chro-

matin alterations. Transducers are brought into action to convey the damage signal to downstream effectors. Phospho-inositide kinase (PIK)-related proteins make up one important group of transducers, with ATM and ATM-Rad3-related (ATR) in mammal. PIK-related kinases target downstream signal transducers by phosphorylating conserved threonines of checkpoint kinases (Chk), as in the case of ATM and ATR, thus initiating cell-cycle arrest to give the cell time to repair the damage. Among the different physiological functions of diphtheria toxin-like ADP-ribosyltransferase (ARTDs) family members, the enzymatic activation of ARTD1 (originally called PARP1) is primarily connected with DNA damage responses and apoptosis. In response to DNA breaks and reactive oxygen species, caused by oxidative stress (e.g. H2O2), ARTD1 forms polymers of ADP-ribose (PAR) and modifies itself and trans-ribosylates other proteins involved in DNA-packaging (histones). Due to its association with XRCC1, which is a known member of the base excision repair pathway, ARTD1 was suggested to be a sensor of single strand breaks *in vivo*. Formed polymers have a very short life time, due to their degradation by poly(ADP-ribose) glycohydrolase (PARG). The signaling function of the formed polymer *in vivo* is currently not understood. The aim of this study is to analyze genotoxic stress-induced proteome changes in an unbiased manner using reverse protein microarrays. First we determined the sub-lethal dose of H2O2 or ionizing radiation (IR) using MRC-5 cells (primary human lung fibroblasts, isolated from a 14-week-old male fetus). Initial immunoflourescence staining confirmed H2O2-induced PAR-formation in response to 100µM H2O2, which could be prevented by pre-incubation with 1µM ABT-888, an ARTD-specific inhibitor. In a similar fashion, X-ray induced phosphorylation of H2AX at Ser139 (γH2AX), a well-established marker for double strand breaks, was also observed as early as 15min (peaking at 1h) after irradiation with 2Gy. Using reverse protein microarrays and lysates of treated MRC-5 cells, we were able to confirm H2AX phosphorylation in response to IR in a pilot study. In agreement with previous studies, other markers, like p53 phosphorylation at Ser15 (p53-S15p) and Chk2 phosphorylation at Thr68 (Chk2-T68p) were significantly elevated in both IR- and H2O2-dependent manner, thus qualifying protein microarrays for studying genotoxic stress-induced proteome changes. Currently, we are analyzing protein-levels and posttranslational modifications in a system-wide manner after IR- and H2O2-treatment, covering players from all known signaling pathways to identify novel players of PAR-induced signaling and to compare the signaling to IR-induced genotoxic stress signaling.

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Biomarker identification in non-small cell lung cancer (NSCLC) with activity-based proteomics

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Background. Lung cancer is the leading cause of all cancer related deaths and treatment is still suboptimal. Novel biomarkers with a reliable predictive significance which may additionally represent therapeutic targets are therefore of utmost importance. In biomarker discovery studies transcript or protein abundances are typically compared in normal versus disease states. However, crucial changes in enzymatic activities remain undetected. Based on the work of Prof. Cravatt and others, activity-based proteomics has become a promising option to circumvent this limitation. This study aims to establish a robust and high throughput activity-based proteomics platform and to investigate the role of serine hydrolase activity profiles as prognostic biomarkers in lung cancer.

Methods. A fluorophosphate derivate (CAS-Number: 353754-93-5) was used to covalently target serine hydrolases in proteomes derived from human lung adenocarcinoma biopsies and corresponding normal lung tissues (tumor cell content: >50%, TNM-stage: I-IV). Tagged proteins were subsequently affinity purified and analyzed using a directed mass spectrometry based approach (LTQ-FTMS, Thermo Finnigan). Data were qualitatively analyzed using the Mascot 2.2 search engine and Progenesis LC-MS version 2.5 (Nonlinear Dynamics) was employed for relative quantification.

Results. The strategy described above results in the simultaneous qualitative and quantitative analysis of serine hydrolase activities in complex proteomes, thereby representing a valid alternative to activity-based proteomics approaches described so far. The analysis of 40 pairs of fresh frozen malignant and corresponding normal lung tissues in combination with clinical follow-up data led to the identification of two biomarker candidates that have previously not been associated with lung cancer.

Conclusion & Outlook. Based on the results obtained in this study we conclude that activity-based proteomics represents a powerful strategy in the

seek for novel biomarker candidates in human lung adenocarcinoma. Future research will involve data validation with additional samples from our tumor bank using advanced quantitative selected reaction monitoring technology.

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Acquired resistance to temozolomide in glioma cells is mediated by different mechanisms

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Temozolomide is the first chemotherapeutic agent that prolongs the survival of patients with glioblastoma. It affects the proliferation of glioma cells by methylation of the O6 position of guanine. This effect is counteracted by the expression of the DNA repair enzyme O6-methylguanine–DNA methyltransferase (MGMT). MGMT is epigenetically silenced in about 40% of all glioblastomas, which correlates with a better response to alkylating chemotherapy. However, even patients with MGMT promoter methylation suffer from tumor progression or relapse within a short period of time. Here, we aimed at generating glioma cells with acquired resistance to temozolomide and identifying the underlying mechanisms. We report that repetitive exposure of different glioma cells to increasing concentrations of temozolomide leads to increased resistance to the compound as assessed in the paradigms of acute cytotoxicity and clonogenic survival. The cells maintain this resistant phenotype for more than a year after cessation of exposure to the compound. In LN-18 glioma cells that constitutively express MGMT, we found a significant up-regulation of MGMT levels after induction of temozolomide resistance. In contrast, MGMT-negative LNT-229 cells that become resistant display a different expression pattern of several mismatch-repair (MMR) genes in the resistant cells. In summary, we demonstrate that resistance to temozolomide can be induced in glioma cells *in vitro*. MGMT-independent pathways contribute to the development of temozolomide resistance in some glioma cells. These findings propose MMR proteins as mediators of acquired resistance of glioma cells to temozolomide.

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Sirtuin relocation to DNA damage foci triggers epigenetic pattern shift, transposon reactivation and pronounced genome instability

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Expression of transposons can be suppressed by methylation of their DNA. This suppression depends on some classes of interfering RNAs (RNAi) such as PIWI-interacting RNAs (piRNAs), endogenous small interfering RNAs and microRNAs (miRNAs). Having found *in silico* that the frequency of CG and CNG sites in these non-coding RNAs greatly exceeds the general genome level, I have suggested that the interfering RNAs can directly hybridize with target DNA sequences, thereafter involving DNA methyltransferases in methylation *de novo* of cytosine within DNA targets [1]. However, at early stages of embryo development, piRNAs and endo-siRNAs disappear because they initiate repression of the sequences from which precursors of these interfering RNAs were transcribed. Using TargetScan software, it was predicted that transcripts of great number of stage-specific genes are the miRNA targets. Therefore, cell differentiation requires repression of some miRNA genes. This makes possible reactivation of transposons in the cells which have lost interfering RNA that recognize sequences of these transposons [2]. Transposons can damage various cell genes, including genes responsible for cell cycle arrest and apoptosis. Moreover, in order to interact with DNA damage sites sirtuin SIRT1, a histone deacetylase, dissociates from heterochromatin and relocates to DNA damage foci. This leads to epigenetic inactivation of antiproliferative and proapoptotic genes even if their damaged sequences have been repaired successfully. In the case of severe DNA damage, SIRT1 can leave heterochromatin sequences entirely. This causes initial shift in epigenetic pattern that can sometimes lead to reactivation of silent oncogenes and antiapoptotic genes without their mutation or translocation. Furthermore, derepression of other transposons becomes possible as a result of sirtuin relocation. As a result, a positive feedback between DNA damage and transposon reactivation establishes, which can rapidly lead to dramatic increase of DNA damage level and genome destabilization. Initial epigenetic shift as well as transposons can also affect the miRNA gene loci. As each miRNA impairs the expression of many genes, including genes of other miRNAs, illegitimate activation or repression of some miRNA genes leads to the total reorganization of epigenetic pattern in transforming cells through the RNAi-dependent DNA methylation. Therefore, the cells begin to express a distorted gene pattern. Illegitimate miRNA expression can directly repress stage-specific genes; thereby cells can lose the normal cytokine susceptibil-

ity. As a result, the course of cell differentiation proves to be complicated, requiring a high concentration of cytokines, or appears to be impossible at all, and transformed cells proliferate and accumulate, forming a tumour. Also, it is plausible, that the alterations in miRNA set allow expression of cell oncogenes and antiapoptotic genes as well as contribute to repression of proapototic, antimetactatic and stage-specific genes. In particular, using TargetScan software it was predicted that transcripts of genes coding proliferative signal pathway components c-Myc and Ras carry targets of miRNAs let-7, let-7a, miR-145, miR-320 and miR-31. miRNAs miR-15, miR-16 and miR-181 silence antiapoptotic gene bcl-2. These miRNAs are usually downregulated in cancer cells. Upregulated miRNA miR-21 inhibits a gene coding tumor suppressor protein pTEN and thus derepresses the PI-3K/Akt antiapoptotic pathway.

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Expression of tumor-promoting Cyr61 is regulated by hTRA2-beta1 and acidosis

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Cysteine rich 61 (Cyr61) is a matricellular protein displaying a remarkable diversity of multiple cellular activities and biological functions involved in significant physiologic and pathologic processes. Cyr61 is an important player in tumor progression, promoting neovascularisation and metastasis. Recently, we showed that Cyr61 pre-mRNA undergoes a specifically-regulated retention of intron 3 during its alternative splicing process as an on/off switch for its biological function (1). Hypoxia leads to increased expression of the intron free splice variant, the solely transcript generating active Cyr61 protein. In this work, we now identify extracellular acidosis as a potent modulator of Cyr61 alternative splicing pattern. In our functional experiments, we observed that acidosis was capable of inducing similar effects on Cyr61 splicing to those caused by hypoxia. Intriguingly, splicing factor hTRA2-beta1 displayed an opposite effect on Cyr61 alternative splicing pattern than hypoxia or acidosis. Considering that, nuclear hTRA2-beta1 protein expression was markedly reduced under hypoxic and acidic conditions. Our findings strongly support the hypothesis of a specific regulation of Cyr61 alternative splicing by the splicing factor hTRA2-beta1. In keeping with these conclusions, we show that hTRA2-beta1 can specifically bind a 'GAAG' motif in Cyr61 exon 3 RNA, that the splicing factor displays acidosis-dependent protein localization in cellular compartments, and that shRNA-mediated hTRA2-beta1 knockdown triggers the same effects on Cyr61 alternative splicing like acidosis or hypoxia, respectively. Our findings might represent auspicious approaches to new vistas in anti-cancer immunotherapies.

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Proteomic identification and quantification of glycoprotein biomarker candidates in malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) is a highly lethal neoplasm of the pleura caused by asbestos exposure. Recent therapeutic advances (Weder, Stahel et al.) have raised growing interest in the identification of serum tumor-markers for the early and correct diagnosis of MPM. In our approach, we suggest the investigation of the MPM cell surface proteome (surfaceome) as a source of new MPM marker-candidates, followed by verification in the serum via selected reaction monitoring (SRM). To unravel the MPM surfaceome, we investigated four MPM primary cell lines via the cell surface capturing technology (Wollscheid et al.), identifying 360 MPM cell surface N-glycoproteins (protein-probability > 0.9). Among them we observed classical MPM markers like mesothelin and N-cadherin. For the selection of MPM

marker-candidates, we compared the MPM surfaceome with a control surfaceome derived from two pleural and two lung adenocarcinoma cell lines. Label-free relative quantitation revealed 70 N-glycoproteins potentially overregulated in MPM together with additional 17 N-glycoproteins potentially specific for MPM. Using the recently developed human synthetic N-glycoproteome (Picotti et al. ; Aebersold et al., in preparation), we are currently investigating by SRM the detectability of those MPM marker-candidates in serum-samples collected at the mesothelioma Biobank of the laboratory of Molecular Oncology (Clinic of oncology, University Hospital Zurich). Promising candidates are further investigated by quantitative SRM between MPM patients and control groups.

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Hierarchy and immunodominance of CD8+ T cell responses to different tumor-associated antigens in patients with hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide with only limited treatment options available to date. Immunotherapy is a novel approach currently under investigation. For a better understanding of the natural immmune response against HCC, we investigated the frequency and immunodominance of CD8+ T cell responses to four antigens expressed by HCC. We used overlapping peptides spanning the entire length of four prominent antigens derived from HCC, specifically alpha-fetoprotein (AFP), Glypican-3, melanoma-associated gene A1 (MAGE-A1) and NY-ESO-1, to stimulate unspecifically expanded CD8+ T cells derived from blood, liver and tumor material of HCC patients (n=60) and controls (n=19). Our analysis revealed that CD8+ T cell responses to all four antigens were present in blood, liver and tumors of HCC-patients without a clear enrichment at the tumor site. Overall, tumor-specific responses were found in 85 % of HCC patients but only in 21 % of controls and thus significantly more frequently in HCC patients (p=0.0001). Interestingly, we observed a clear hierarchy of antigen-specific CD8+ T cell responses. Indeed, 33 of 60 patients recognized peptides from NY-ESO-1, 23 patients recognized peptides from AFP, 22 patients those from Glypican-3 and 14 patients those from MAGE-A1. Of note, the responses to Glypican-3, MAGE-A1 and NY-ESO-1 were each governed by single immunodominant epitopes (two for Glypican-3 and MAGE-A1, one for NY-ESO-1), two of which could be newly identified. In contrast, responses to AFP were spread across the whole protein and targeted 14 different epitopes without clear immunodominance. Two AFP-peptides were recognized more frequently, one of those was also newly identified. Interestingly, the immunodominance was also reflected in the frequency of antigen-specific CD8+ T cells in the individual patients. Indeed, Glypican-3 and NY-ESO-1 induced significantly stronger responses than AFP (p=0.0078 and 0.0009, respectively). Altogether, the immune response against HCC is characterized by a clear hierarchy of antigen-specific immune responses, especially targeting NY-ESO-1. Interestingly, the CD8+ T cell response was

clearly dominated by single epitope-specific responses, some of which were newly identified. This was not the case for AFP that induced an unfocused response without a clear immunodominance. These data support further investigations into immunotherapeutic approaches for the therapy of HCC that should not be limited to AFP alone.

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The combination of psoralen and UVA irradiation has an anti-growth effect on human bladder cancer cell lines

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Introduction: Although immunotherapy and chemotherapy are the most commonly used intravesical treatments for non-muscle-invasive bladder cancer (NMIBC), progression and recurrence rates are high. So far, several attempts have been made to introduce a safer and more efficient treatment. Psoralen (a photoreactive chemical substance) treatment followed by ultra violet A (UVA) irradiation (PUVA) has been previously used to treat cutaneous T-cell lymphoma, chronic graft-versus-host disease, and psoriasis. UVA radiation creates covalent bonds between psoralen molecule and pyrimidine bases of DNA resulting in interstrand cross-links, which block DNA replication, thereby inhibiting cell proliferation. In addition, PUVA therapy causes apoptosis and local immune reaction. The aim of this study was to evaluate the anti-growth effects of PUVA treatment on human bladder cancer cell lines as the first step of further investigations, in order to consider it as a new candidate for NMIBC treatment.
Materials and Methods: Human bladder cancer cell lines (T24, grade III and RT4, grade I) were cultured *in vitro*, treated with either 250 or 500ng/ml 8-methoxypsoralen (8-MOP, a natural psoralen metabolite). Cells were then irradiated with UVA (365 nm) at different doses (0, 0.5, 1, 2.5, 4, and 5 J/cm2). After 24-, 48-, 72- or 96-hour PUVA treatment, cells were counted to measure cell growth. MTT assay was performed in order to analyze cell viability and BrdU incorporation assay to evaluate the anti-proliferative effect of PUVA treatment. Cells were also stained with Annexin V-FITC and observed under fluorescence microscope in order to measure the amount of apoptotic cells.
Results: Both T24 and RT4 cells treated with 2.5 J/cm2 UVA and higher doses showed a significant decrease ($P < 0.05$) in the amount of cells after 24 hours, as compared to no UVA-treated cells. After 48 hours, cell lines treated with 1 J/cm2 UVA also showed a significant decrease in the number of cells. In MTT assay of PUVA-treated RT4 cells, treatments with 2.5 J/cm2 UVA and higher doses significantly reduced cell viability after 24 hours in comparison to no UVA treatment, while there were no significant changes with 0.5 and 1 J/cm2 UVA treatment. Then, after 48 hours, cells treated with 1 J/cm2 UVA also revealed a significant reduction in cell viability. In T24 cells, all the UVA doses more than 0.5 J/cm2 UVA induced a significant reduction of cell viability compared to no UVA.
BrdU incorporation assay in both cell lines revealed a significant reduction of cell proliferation 24 hours after PUVA treatment with 1 J/cm2 UVA and higher doses in comparison to no UVA treatment. Fluorescent microscopic observation of cell lines stained using Annexin V-FITC showed an increased number of apoptotic cells among those particularly treated with 2.5, 4, and 5 J/cm2 UVA doses after 24 hours, as compared to no UVA-treated cells.
Conclusions: PUVA treatment has a significant anti-growth effect on human bladder cancer cell lines by inducing cytotoxicity, blocking proliferation and triggering apoptosis in the tumor cells. These results suggest a possible relevant use of PUVA therapy for the treatment of NMIBC.

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Hypoxia-dependent in vitro selection of stem-like cell population in primary human brain tumor cultures

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Objective: It has been recently shown that brain tumors contain a distinct cell population, responsible for initiation of the tumor growth, progression and apparent resistance to commonly used therapeutic modalities (chemo-

and radiotherapy). These so called brain tumor stem cells (BTSC) share many properties with somatic stem cell i.e. self-renewal through asymmetric cell division, the capacity to generate non tumorigenic end cells and the ability to initiate cancers upon orthotropic implantation in experimental animals giving rise to tumors being a phenocopy of the tumor of origin. Here we define *in vitro* conditions and explore the mechanisms resulting in significant increase of the stem cell status of *de novo* generated primary cell lines from patients with glioblastoma (GBM) (n=5) as well as for fetal derived cerebral cortex.

Results: Subject to mild hypoxia (3% oxygen tension) up to one week all cell cultures showed a significant augmentation of stem cell population as reveald by FACS analysis (CD133 as well as Aldefluor) and by quantitative gene expression study (rt PCR for CD133, SOX2, NANOG). CD133 positive fraction increased from 3% to 16% in tumorgenic cultures and up to 61% in case of propagated fetal tissue. In parallel up to 400 fold increase of PROMININ 1 expression in GBM cultures and 7fold in human fetal cortical samples was observed. Proliferation kinetics assessed with MTS assay revealed significantly diminisched growth of cultures cultivated under hypoxia compared to samples propagated under atmospheric oxygen tension. The rt PCR-based assessment of hypoxia treated cultures showed overexpression of genes involved in hypoxia response (i.e HIF1 alpha) as well as increased activity of pro-apoptotic genes (i.e. BAX, BNIP3). Interestingly, the latter was restricted to CD 133 negative cell fraction. In concordance with this data the Western-Blot analysis showed the presence of activated Caspase-3 - a dominant player in initiation of programmed cell death – and BNIP3 exclusively in CD133 negative population.

Conclusion: Here we confirm the hypoxia-dependant increase of BTSC in cultivated primary brain tumor and human fetal cortical samples. This study is, however, to our knowledge the first proof for BTSC resistance against hypoxia-induced apoptosis. Moreover, the fast induction of CD133+ cell population has not been observed in the current study. Therefore, we postulate that the major mechanisms of in vitro hypoxia-dependant increase of BTSC is their lack of susceptibility to oxygen deprivation resulting in better survival and selection under hypoxic culture conditions. Furthermore, our protocol allows the generation of significant numbers of BTSC for further genetic manipulations and ex vivo testing of novel therapeutic strategies.

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Embryonic Lethality of Tdg Deficient Mice Reveals a Function of DNA Repair in the Maintenance of Epigenetic Stability

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Thymine DNA glycosylase (TDG) is a mismatch-directed DNA glycosylase that acts on a broad spectrum of DNA base lesions. Because TDG excises thymine from G•T mismatches, it was proposed to counteract mutagenesis arising from deamination of 5-methylcytosine in mammalian DNA. Physical and functional interactions with transcription factors and chromatin modifiers, however, suggested an involvement in gene regulation. To clarify the biological function of this DNA repair enzyme, we generated a TDG knockout in mouse. This revealed that, unlike other DNA glycosylases, TDG has an essential role in early mouse development. TDG knockout fibroblasts (MEFs) showed significantly impaired gene expression due to imbalanced histone modifications and aberrant CpG methylation in gene promoters. Using an *in vitro* differentiation system we found that such epigenetic aberrations are not observed in TDG deficient embryonic stem cells (ESC) but arise during cell differentiation. We could further show that TDG associates physically with CpG-rich promoters of differentially regulated genes both in ESC and in MEFs. Our data suggest that, as a structural component of gene regulatory complexes TDG supports the establishment and maintenance of active or bivalent chromatin states throughout cell differentiation, thereby using its DNA glycosylase activity to prevent aberrant gene silencing. Thus, the TDG-dependent pathway of DNA base excision has evolved to provide stability to epigenetic states during cell differentiation and development.

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A new diagnostic classifier for Burkitt and Diffuse Large B-Cell Lymphoma predicts outcome

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Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) represent distinct entities among aggressive B-cell non-Hodgkin lymphomas (BCL), each with important clinical and therapeutic consequences. However, there is increased recognition of DLBCLs which share features with BL but deviate with respect to one or more findings and are currently classified in a provisional intermediate group in the WHO classification 2008. To overcome the poor diagnostic reproducibility of this category, we analyzed 242 cytogenetically defined (BCL2, BCL6, MYC translocations) BCL for differential protein expression of selected markers, based on recent transcriptional and gene-expression profile studies. By analyzing primary lymphomas on a tissue microarray, we identified expression of CSE1L and ID3 as being associated with the diagnosis of BL ($p < 0.05$), whereas STAT3 was over-expressed in DLBCL. All three markers were associated with favourable prognosis in DLBCL. Most interestingly, machine learning techniques for classification showed that only the combination of CSE1L, STAT3 and MYC translocation, subsumed under the algorithm called "new FISH classifier", was able to identify patients, initially classified as intermediate between BL/DLBCL, who profited from intensive, BL-like regimens. We present a promising diagnostic algorithm for BCL with additional prognostic and predictive values.

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DNA methylation profiling in melanoma

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Melanoma is the most aggressive skin cancer worldwide. Although melanoma is curable when detected in early stages of progression, patients diagnosed with late stage and metastatic melanomas have very poor survival. The difficulty in treating late stage and metastatic melanoma patients has been attributed to the heterogeneity of melanoma. Our group recently described a model for melanoma heterogeneity(1). However the molecular mechanisms of establishing heterogeneity are currently unknown. To investigate the role of DNA methylation on melanoma heterogeneity we performed DNA methylation profiling of 7 melanoma cell cultures established from 7 patient melanoma biopsies by methylated DNA immunoprecipitation microarray analysis using the Nimblegen 720K Human CpG Refseq promoter array. The DNA methylation profile of the 7 melanoma cell cultures could be distinguished into two distinct cohorts. Preliminary analysis of this dataset identified genes involved in the WNT signaling pathway such as WNT5A, TCF4, and FZD2 to be differentially methylated between the two cohorts. Expression levels of these genes correlated with the methylation status of the promoter. These results suggest that DNA methylation might be a mechanism which regulates WNT signaling in melanoma.

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A ZEB1/microRNA-200 feedback loop controls epithelial to mesenchymal transition and stemness properties

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Invasion and metastasis of carcinomas is promoted by the aberrant activation of the embryonic epithelial to mesenchymal transition (EMT) program at the invasive front, which triggers cellular mobility and subsequent dissemination of tumor cells. Strikingly, metastases often recapitulate the differentiated phenotype of the primary tumor, indicating a mesenchymal-epithelial re-transition. We recently described that the EMT-activator ZEB1 is a crucial promoter of metastasis formation and demonstrated that ZEB1 inhibits expression of the miR-200 family of microRNAs, whose members are strong inducers of epithelial differentiation.
We discovered that ZEB1 not only promotes tumor cell dissemination, but is also necessary for the tumor initiating capacity of breast, pancreatic and colorectal cancer cells and mediates drug resistance. We showed that ZEB1 represses the expression of microRNA-203, and that miR-200 family members and miR-203 cooperate to suppress the expression of pluripotency- or stemness-associated factors in tumor cells, as demonstrated for the polycomb group protein BMI1. Furthermore, we linked ZEB1 and its cancer promoting properties to Notch activation. Notch signalling is important for development and tissue homeostasis and activated in many human cancers. We showed that miR-200 family members target the Notch pathway components Jagged1 (JAG1) and the mastermind-like co-activators MAML2 and MAML3, thereby mediating enhanced Notch activation by ZEB1. These findings could be an explanation for increased Notch signaling in tumors, where mutations in Notch pathway genes are rare. In conclusion we propose that ZEB1 links activation of EMT, maintenance of stemness and acquisition of drug resistance and provide the underlying molecular mechanism: The suppression of stemness-inhibiting microRNAs by EMT-activators. These data indicate that EMT-activators control cell motility, stemness and drug resistance and support that ZEB1 is a promoter of mobile, migrating cancer stem cells. Thus targeting the ZEB1 – miR-200 feedback loop might be a promising treatment option for fatal tumors like pancreatic cancer.

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Looking for a stemness signature in prostate cancer

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Tumor initiation and growth might be driven by a rare population of cells endowed with stem-properties and therefore defined as cancer stem cells (CSC)(1). CSC may share properties of normal stem cells, such as self-renewal and differentiation potential, and molecular pathways typically used by pluripotent embryonic stem cells (2). Moreover, recent evidences also indicate an inappropriate activation of embryonic regulation genes during transformation. In this study we have therefore evaluated the expression of known pluripotency-associated genes in prostate cancer (PCA) and benign prostatic hyperplasia (BPH). Specific gene expression was evaluated by quantitative real time PCR (qRT-PCR) in PCA cell lines and tissues obtained from BPH and PCA. Gene expression was also verified at the protein level by using specific antibodies in immunofluorescence and immunohistochemistry assays. Furthermore a tissue micro-array (TMA) including over 600 PCA samples was also stained with specific antibodies.
In a large majority of samples, irrespective of their malignant nature, detectable expression of Sox2, Oct4, Nanog, c-Myc, and Klf4 genes was observed. However, c-Myc and Klf4 were found to be expressed to significantly higher extents in PCA than in BPH tissues. Klf4 protein was undetectable in BPH, but specific staining was observed in PCA tissues, and, in particular, in the cytoplasm of cells expressing chromogranin A, a neuroendocrine marker. Cytoplasmic co-localization was confirmed by immunofluorescence and a

highly significant correlation between the expression of the two markers was also observed upon TMA staining.
These data indicate that a specific stemness signature is detectable in PCA cell lines and prostatic tissues. Moreover, neuroendocrine cells, a cell population exquisitely expanded in advanced PCA, display high expression of Klf4 transcription factor. Conspicuously, cytoplasmic localization of this protein appears to represent a specific feature of PCA neuroendocrine cells.

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Effects of CD44 down-regulation on in vitro metastatic properties of highly metastatic human oteosarcoma 143B/LacZ cells

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Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents with a high propensity for metastasis and therefore poor prognosis. Despite the significant clinical improvements made over the past decades through the combined chemotherapeutic and surgical treatment, patients with metastatic or recurrent disease continue to have a very poor prognosis, with a 5-year survival rate of 10-20% (1). Therefore, it is important to better understand the molecular mechanisms of osteosarcoma progression and metastasis in order to develop more effective therapeutic strategies that ultimately improve the survival of these patients.
CD44 is frequently found overexpressed in tumour cells and has been implicated in metastasis. It is a cell-cell and cell-matrix adhesion molecule and the principal receptor for hyaluronan (HA), a major component of the extracellular matrix. CD44 is a transmembrane glycoprotein with biological functions in development, inflammation, haematopoiesis, wound healing, immune response and tumour progression (2).
Western blot analysis revealed increased CD44 expression in 3 out of 4 highly metastatic human OS cell lines compared to the parental cells with lower metastatic potential. Therefore, we investigated the effects of stable shRNA-mediated CD44 down-regulation on in-vitro metastatic properties of the highly metastatic human 143-B/LacZ OS cell line in adhesion, migration and soft agar colony formation assays.
143-B/LacZ cells with stably down-regulated CD44 expression were generated by retroviral infection. The efficiency of CD44 down-regulation was examined by Western blot analysis of cell lysates. The CD44 protein expression in cells infected with the CD44 specific shRNA was almost abolished compared to the cells infected with the control shRNA and the empty vector (EV). The cell surface expression assessed by immunocytochemistry was similarly suppressed. Stable down-regulation of CD44 did not affect cell proliferation and adhesion to endothelial cells, but it resulted in a 71% decrease in the adhesion to HA, a 58 % decrease in the migration rate in a trans-filter migration assay, and a 30% decrease in the cells' capacity for anchorage-independent growth in soft agar. These results point to important roles of CD44 in cellular processes contributing to the metastatic activity of 143-B/LacZ cells.
The effect of CD44 silencing on tumour progression and metastasis formation will be further analysed in vivo in an intratibial xenograft osteosarcoma mouse model.

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Charles Rodolphe Brupbacher Stiftung

Charles Rodolphe Brupbacher Foundation

Mme. Frédérique Brupbacher

Portrait by Peter Cerutti

Charles Rodolphe Brupbacher Stiftung

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

Die Stifterin

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

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

Charles Rodolphe Brupbacher Foundation

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

The Founder

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

The Executive Board of the University of Zurich appointed Mrs. Frédérique Brupbacher in 2005 as a permanent Guest of Honor of the University, in appreciation of her altruism and her engagement for the cancer research. Through her personal committment, cancer research in Zurich has been significantly strengthened. President Jacques Chirac of France elected her to Chevalier de la Légion d'Honneur.

Charles Rodolphe Brupbacher
1909 - 1987

Charles Rodolphe Brupbacher wurde am 5. Februar 1909 in Zürich als Bürger von Wädenswil geboren. Sein Vater, C.J. Brupbacher, war Inhaber einer Privatbank am Paradeplatz. Die Mutter, geborene Französin, legte grossen Wert auf eine zweisprachige Erziehung des Sohnes. Dies erklärt auch seine lebenslange, enge Beziehung zu Frankreich, zu dessen Geschichte und Kultur und seine dauernde, grosszügige Unterstützung der Ecole française und der Alliance française in Zürich. Sein jahrzehntelanger Einsatz für die Anliegen der französischen Kultur wurde mehrfach durch die jeweiligen Staatspräsidenten geehrt:

1961 Präsident Charles De Gaulle
Ernennung zum Chevalier de la Legion d'Honneur
1973 Präsident Georges Pompidou
Ernennung zum Officier de la Legion d'Honneur
1979 Präsident Valéry Giscard d'Estaing
Ernennung zum Commandeur de l'Ordre National de Merite

Schon früh zeigte sich bei Charles Rodolphe Brupbacher eine ausgesprochene Sprachbegabung; er beherrschte fünf Sprachen fliessend. Als musikalisches Wunderkind mit dem absoluten Gehör widmete er sich der Interpretation klassischer Musik und bedauerte zeitlebens, dass er auf eine Ausbildung als Konzertpianist verzichten musste. Charles Rodolphe Brupbacher besuchte die Schulen in Zürich und Paris.

Charles Rodolphe Brupbacher was born on February 5, 1909 in Zurich, as a citizen of Wädenswil. His father, C.J. Brupbacher, owned a private bank at the Paradeplatz. His mother, a French citizen, placed great importance on a bilingual education for her son. This explains his lifelong, close relationship with France, its history and culture. This is also reflected by his continuous and generous support of the École française and the Alliance française in Zurich. Several French Presidents honoured his commitment to French cultural issues:

1961 President Charles De Gaulle
Election to Chevalier de la Legion d'Honneur
1973 President Georges Pompidou
Election to Officier de la Legion d'Honneur
1979 President Valéry Giscard d'Estaing
Election to Commandeur de l'Ordre National de Merite

At an early age, Charles Rodolphe Brupbacher showed a distinct talent for languages, and he spoke five of them fluently. As a musical prodigy with absolute pitch, he devoted himself to the interpretation of classical music. He regretted throughout his life that he had not been able to receive an education as a concert pianist. Charles Rodolphe Brupbacher attended schools in Zurich and Paris.

Mit 18 Jahren musste er auf Verlangen seines Vaters die Ausbildung am Gymnasium in Zürich und Paris aufgeben und eine Banklehre absolvieren. Anschliessend besuchte er ab 1929 immer wieder die Vereinigten Staaten, sowie Lateinamerika und trat so in Beziehung zu grossen Persönlichkeiten in führender Stellung.

Nach seiner Rückkehr in die Schweiz gründete er, als damals jüngster Bankier, mit 24 Jahren die auf Vermögensverwaltung spezialisierte Bank «Affida» am Paradeplatz in Zürich. Sein Erfolg war in hohem Masse seinen Geschäftsprinzipien zu verdanken. Dazu gehörte der Aufbau eines Informationsnetzes, welches ihn mit den wichtigsten finanziellen und politischen Zentren verband. Von grosser Bedeutung waren dabei seine detaillierten Kenntnisse der internationalen Rechtsprechung, der Nationalökonomie und ganz speziell auch von Währungsfragen. Nach 40jähriger Tätigkeit verkaufte er die Affidabank an die Schweizerische Kreditanstalt (Credit Suisse).

Auf Grund seiner umfassenden Kenntnisse wurde Charles Rodolphe Brupbacher 1938 von Prof E. Böhler in die Gruppe für Konjunkturbeobachtung der Eidgenössischen Technischen Hochschule (ETH) berufen. Als deren Mitglied nahm er auch an Besprechungen kriegswirtschaftlicher Probleme in Bern teil.

Als anerkannter Fachmann in Währungsfragen wurde Charles Rodolphe Brupbacher nach dem Kriege als einziger Beobachter aus der Schweiz zu den internationalen Währungskonferenzen eingeladen. Seine persönlichen Beziehungen zu wichtigen Politikern in den USA erlaubten es ihm, durch jahrelange, zähe Verhandlungen grosse schweizerische Guthaben zu deblockieren.

Auch bemühte sich Charles Rodolphe Brupbacher intensiv um die Probleme, welche sich bei dem Wiederaufbau der Montanindustrie zwischen Deutschland und den Alliierten entwickelt hatten. In diesem Zusammenhang wurde er von der französischen Regierung und der Regierung von Nordrhein-Westfalen zur Teilnahme an dem Treffen anlässlich der ersten Reise von General de Gaulle nach Deutschland eingeladen.

Schon im Jahre 1963 hat Charles Rodolphe Brupbacher an der ETH eine Stiftung zur Unterstützung von Studierenden auf dem Gebiet der Sozialwissenschaften gegründet, die seither laufend Stipendien vergibt.

Charles Rodolphe Brupbacher starb am 1. Januar 1987 und hinterliess seine Ehefrau Frédérique, die er 1953 geehelicht hatte.

At the age of 18, however, he had to give up his education at the Gymnasium (College) to undertake a banking apprenticeship. He visited the United States and Latin America in 1929 and frequently thereafter: first, for the purpose of training; later, to keep himself informed.

At the Paradeplatz in Zurich, at the age of only 24, he established the «Affida Bank», which specialized in asset management. His success was largely due to a committment to personal business integrity. His achievements included the setting-up of an information network that connected him with important financial and political centres. His detailed knowledge of international commercial law, of national economics and, especially, of currency policy were great assets. After 40 years, he sold the «Affida Bank» to Credit Suisse.

Based on his detailed knowledge, Charles Rodolphe Brupbacher was invited by Professor E. Böhler in 1938 to join a select group formed at the Swiss Federal Institute of Technology (ETH), which met to monitor the economy. As a member, he often took part in discussions in Bern of wartime economic problems.

As a recognised expert in monetary policy, Charles Rodolphe Brupbacher was the only observer from Switzerland to be invited after the war to the international currency conferences. His personal relationship with prominent politicians in the United States enabled him, through years of negotiations, to release major Swiss assets.

Charles Rodolphe Brupbacher also helped to attenuate problems which had developed between Germany and the Allies regarding the restoration of the coal and steel industry. In this context, he was invited by the Government of France and by the State of North Rhine-Westphalia to participate in the meeting on the occasion of General de Gaulle's first visit to Germany.

Already in 1963, Charles Rodolphe Brupbacher established a Foundation at the ETH with the objective of supporting students in the field of social sciences. Since then, the Foundation has continuously granted scholarships.

Charles Rodolphe Brupbacher died on January 1, 1987, survived by his wife Frédérique whom he married in 1953.

Stiftungsrat

Der Stiftungsrat verwaltet die Stiftung und vertritt sie nach aussen. Er trifft die Entscheide über Preisverleihungen und die begleitenden wissenschaftlichen Symposien.

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

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

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