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Wissenschaftliches Symposium
2009

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Inhalt

Charles Rodolphe Brupbacher Preis für Krebsforschung 2009

Programm der Preisverleihung 9

Nubia Muñoz 11
- Laudatio 11
- CV und Publikationen 12
- Festvortrag 15

Sir Richard Peto 19
- Laudatio 19
- CV und Publikationen 20
- Festvortrag 23

Bisherige Preisträger 25

Symposium 2009 27
- Programm 28
- Abstracts der Referenten 30
- Poster Abstracts 50

Charles Rodolphe Brupbacher Stiftung 113
- Die Stiftung 114
- Charles Rodolphe Brupbacher 115
- Stiftungsrat 117
- Wissenschaftlicher Beirat 118
- Stiftungsadresse 119

Adressen 120

Index 123

Contents

Charles Rodolphe Brupbacher Prize for Cancer Research 2009

Program of the Award Ceremony 9

Nubia Muñoz 11
- Laudatio 11
- CV and Publications 12
- Lecture 15

Sir Richard Peto 19
- Laudation 19
- CV and Publications 20
- Award Lecture 23

Previous Laureates 25

Symposium 2009 27
- Program 28
- Abstracts of Invited Speakers 30
- Poster Abstracts 50

Charles Rodolphe Brupbacher Foundation 113
- The Foundation 114
- Charles Rodolphe Brupbacher 115
- Foundation Board 117
- Scientific Advisory Board 118
- Address of the Foundation 119

Address List 120

Index 123

Preisverleihung

Charles Rodolphe Brupbacher Preis für Krebsforschung 2009

Award Ceremony

Charles Rodolphe Brupbacher Prize for Cancer Research 2009

Charles Rodolphe Brupbacher Preis für Krebsforschung 2009

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 2009 wird verliehen an:

Nubia Muñoz, Lyon, Frankreich

Sir Richard Peto, Oxford, Vereinigtes Königreich

Charles Rodolphe Brupbacher Prize for Cancer Research 2009

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 2009 Prize are:

Nubia Muñoz, Lyon, France

Sir Richard Peto, Oxford, United Kingdom

Charles Rodolphe Brupbacher Preis für Krebsforschung

Universitätsspital Zürich, Grosser Hörsaal Nord, Frauenklinikstrasse 10, 8091 Zürich
Donnerstag, 12. Februar 2009, um 17:00 Uhr

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

Frédéric Chopin, Fantasie Impromptu

Laudationes
Prof. Dr. Nubia Muñoz
Prof. Sir Richard Peto

durch

Prof. Dr. D. Maxwell Parkin
Prof. Dr. Paul Kleihues

Preisverleihung
Frau Frédérique Brupbacher, Präsidentin der Stiftung

Robert Schumann, Kinderszenen, Träumerei und Wichtige Begebenheit

Referate
Prof. Dr. Nubia Muñoz

Franz Liszt, Vallée d` Obermann

Prof. Sir Richard Peto

Ludwig van Beethoven, Sonate Op. 2 Nr. 3 in C-Dur Allegro con brio

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 12th, 2009, 17:00 pm

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

Frédéric Chopin, Fantasie Impromptu

Laudationes
Prof. Dr. Nubia Muñoz
Prof. Sir Richard Peto

by

Prof. Dr. D. Maxwell Parkin
Prof. Dr. Paul Kleihues

Awards
Mrs. Frédérique Brupbacher, President of the Foundation

Robert Schumann, Kinderszenen, Träumerei und Wichtige Begebenheit

Lectures
Prof. Dr. Nubia Muñoz

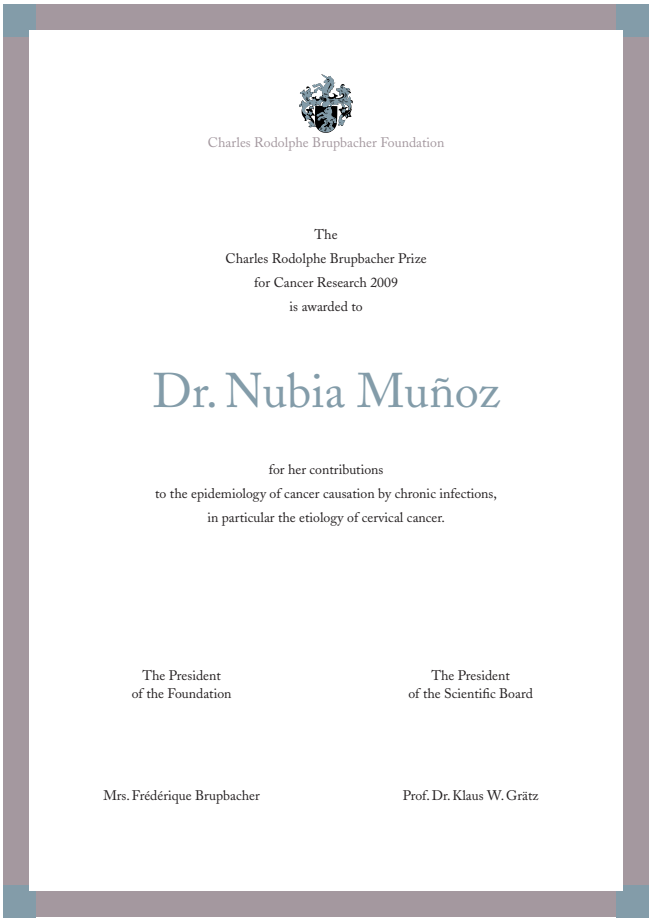
Franz Liszt, Vallée d'Obermann

Prof. Sir Richard Peto

Ludwig van Beethoven, Sonata Op. 2 Nr. 3 in C-Dur Allegro con brio

Final address
Prof. Dr. Klaus W. Grätz

Apéro



Laudatio

D. Maxwell Parkin

Dr Muñoz career began as a pathologist in her native Colombia, in the department of her mentor Professor Pelayo Correa. Her professional interest soon moved to the field of epidemiology, however, and she received her training in the discipline at the US National Cancer Institute, and Johns Hopkins University. Her research first focussed on gastric cancer, with a series of studies following up the observations of Lauren on the differing aetiology and geographic distribution of intestinal and diffuse types of gastric carcinoma. This work took her to the International Agency for Research on Cancer (IARC) in Lyon in the 1970's, where she worked with Dr Callum Muir and Dr Nick Day, before taking charge of her own research unit in the mid 1980's. Her main research interest at that time was in oesophageal cancer, and particularly the reasons for its striking geographic distribution. Work with Nick Day in Iran in the pre-revolutionary days was followed by a series of studies in high-risk areas of China, including an elaborate intervention study with micronutrients (β-carotene, riboflavin and zinc). Her group also conducted a series of studies on the epidemiology of hepatocellular carcinoma in Asia and Africa.

But, even by this time, Dr Muñoz had an ongoing interest in cancer of the uterine cervix. The epidemiology of this cancer had been much studied in the 1960's and 70's, and, in a review in 1976, Dr Munoz concluded *"The evidence suggests that a venereally transmitted virus and/or hormonal factors are involved in the etiology of cervical cancer"*. This was before the work of Prof Harald zur Hausen in the early 1980's, detecting the presence of human papilloma virus in tumour tissue (for which he was later awarded the Nobel prize). At that time, there was some scepticism concerning the role of HPV infection. In an important review, published in 1988, Dr Munoz wrote:

"The human papillomavirus has emerged over the past decade as the leading candidate to be the sexually transmitted aetiological factor in cervical cancer. Although it appears that papillomavirus types 16 and 18 are associated with a higher risk of advanced cervical neoplasia, most of the evidence comes from studies which do not satisfy basic epidemiological requirements, and are

therefore difficult to interpret……. On the basis of the existing studies, one is forced to conclude that, while experimental data suggest an oncogenic potential for HPV, the epidemiological evidence implicating it as a cause of cervical neoplasia is still rather limited".

From her unit at IARC Dr Muñoz began to lead a major programme of research. This began with an international series of case-control studies using modern laboratory techniques that demonstrated that HPV infection by certain genotypes of HPV is one the strongest cancer risk factors ever found. Subsequent work produced precise estimates of relative risks that permitted defining the HPV genotypes that had to be targeted for prevention. At the same time, the International Biological Study of Cervical Cancer (IBSCC) identified the HPV types associated with tumours occurring in different parts of the world. By 1999, it was possible to assert that HPV infection should considered a necessary cause of cervix cancer. Dr Muñoz convinced the IARC that the role of HPVs should be evaluated in one of the authoritative Monograph series on carcinogenicity evaluation. In 1995, HPVs 16 and 18 were classified as "Group 1, Human Carcinogens". This monograph was an important stimulus to the development of HPV tests with the aim of improving traditional cervical cancer screening, which had hitherto relied upon cytology examination using the Pap test. Perhaps even more importantly, the Monograph gave pharmaceutical companies the evidence needed to take the financial risks in developing and field-testing candidate HPV vaccines. The end result is that there are now two new fronts for cervical cancer prevention: HPV vaccination and improved screening with HPV tests, all originated from Dr Muñoz' vigorous and relentless leadership on the epidemiology front grounded on the pioneer work by zur Hausen.

Dr Muñoz retired from her post at IARC in 2002, but has continued to play a major role as Emeritus Scientist, National Cancer Institute of Colombia, acting as advisor to vaccine companies in conducting relevant trials the results of which have influenced policy with respect to immunisation worldwide. She has been the recipient of many awards and prizes, including the Premio Atlántico de Investigación del Cáncer (2004), The Outstanding Epidemiologist Award (Society for Epidemiologic Research, 2006), and the International Epidemiological Association's Sir Richard Doll's Prize in Epidemiology (2008).

Dr. Nubia Muñoz

Curicculum vitae and Publications

Place of birth	Cali, Colombia
Home address	24, Quai Fulchiron 69005 Lyon (France) Telephone: +33 478429021
Current position	Emeritus Professor, National Cancer Institute, Bogota, Colombia Visiting Scientist, Catalan Institute of Oncology, Barcelona, Spain

Graduate and Postgraduate Education

1958 - 1964	University of Valle Faculty of Medicine Cali, Colombia Degree: Doctor of Medicine and Surgery
1964 - 1967	University of Valle Faculty of Medicine Cali, Colombia Degree: Board of Pathology
1967 - 1968	Fellow in the Department of Pathology National Cancer Institute National Institutes of Health Bethesda, MD, USA

1968 - 1969	Postgraduate student School of Public Health Johns Hopkins University Baltimore, MD, USA Degree: Master of Public Health Subject: Cancer Epidemiology

Professional Experience

1969 – 1970	Research Associate (Training) Unit of Epidemiology International Agency for Research on Cancer (IARC), Lyon, France
1970 - 1972	Scientist Unit of Biological Carcinogenesis IARC, Lyon, France
1972 - 1973	Visiting Scientist Laboratory of Virology Department of Pathology National Cancer Institute National Institutes of Health Bethesda, MD, USA
1973 - 1976	Epidemiologist Unit of Biological Carcinogenesis IARC, Lyon, France
1976 - 1979	Epidemiologist Unit of Interdisciplinary Programme and International Liaison IARC, Lyon, France
1984 - 1986	Epidemiologist Unit of Biostatistics and Field Studies IARC, Lyon, France
1986 – 2000	Chief Unit of Field and Intervention Studies IARC, Lyon, France
2000 - 2001	Consultant. Emeritus Scientist. Unit of Field and Intervention Studies IARC, Lyon, France
2000 –to date	Emeritus professor at the NCI of Colombia Consultant Epidemiology and Cancer Registration Unit Catalan Institute of Oncology, Spain

Honors and Awards

1967 - 1969	IARC fellowship 1967-1969, NCI, Bethesda, MD, and Johns Hopkins University, Baltimore, MD
1972-1973	Visiting Scientist, National Cancer Institute, Bethesda, MD, USA
1992	Distinguished Alumna Award of the Johns Hopkins University
2001	Honorary Member of the National Academy of Medicine (Colombia)
2002	Emeritus Scientist of the National Cancer Institute of Colombia
2004	Premio Atlantico del Cancer, ICIC, Canarias
2004	Member of the Johns Hopkins Society of Scholars
2004	Emeritus Scientist of the Universidad del Valle, Colombia
2004 - 2007	Member of External Committte to Evaluate the "Red de Cancer y de la Red de Epidemilogia y Salud Publica de Espana"
2006	"Distinguished epidemiologist award" by three Epidemiology Societies of North America: SER, ACE, APHA-EPI and IEA, during the 2006 Congress of Epidemiology in Seattle
2006	Sciences Award from Fundacion Alejandro Angel Escobar. Bogota, Colombia
2007	Doctora Honoris Causa en Ciencias Biomedicas by the University of Antioquia, Medellin, Colombia
2007	Award "Luis Patino Camargo" from the Colombian Association of Infectious Diseases, Bogota, Colombia
2005 - 2007	Member of the World Health Organization (WHO) HPV Global Advisory Board
2008	Award "IEA Sir Richard Doll" at the World Congress of Epidemiology, Porto Alegre, Brazil

Selected Publications

Muñoz, N. (1976) Model systems for cervical cancer.
Cancer Res., 36, 792

Muñoz, N., Bosch, X. & Kaldor, J.M. (1988) Does human papillomavirus cause cervical cancer? The state of the epidemiological evidence. Br. J. Cancer, 57, 1 5.

Muñoz, N., Bosch, F.X., de Sanjosé, S., Tafur, L., Izarzugaza, I. Gili, M., Viladiu, P., Navarro, C., Martos, C., Ascunce, N., Gonzalez, L.C., Kaldor, J.M., Guerrero, E., Lörincz, A., Santamaria, M., Alonso de Ruiz, P., Aristizabal, N. & Shah, K. (1992) The causal link between human papillomavirus and invasive cervical cancer: a population based case control study in Colombia and Spain. Int. J. Cancer, 52, 743 49.

Muñoz, N., Bosch, F.X., de Sanjosé, S., Vergara, A., del Moral, A., Muñoz, M.T., Tafur, L., Gili, M., Izarzugaza, I., Viladiu, P., Navarro, C., Alonso de Ruiz, P., Aristizabal, N., Santamaria, M., Orfila, J., Daniel, R.W., Guerrero, E. & Shah, K.V. (1993) Risk factors for cervical intraepithelial neoplasia grade III/carcinoma in situ in Spain and Colombia.
Cancer Epidemiology, Biomarkers & Prevention, 2, 423 31.

Muñoz, N., Crawford, L. & Coursaget, P. (1995) HPV vaccines for cervical neoplasia [Meeting report]. Lancet, 345, 249.

Muñoz, N., Castellsagué, X., Bosch, F.X., Tafur, L., de Sanjosé, S., Aristizabal, N., Ghaffari, A.M. & Shah, K.V. (1996) Difficulty in elucidating the male role in Colombia, a high-risk area for cervical cancer. J. Natl Cancer Inst., 88, 1068-75.

Muñoz, N. (2000) Human papillomavirus and cancer: the epidemiological evidence. J. Clin. Virol., 19, 1-5.

Muñoz, N., Franceschi, S., Bosetti, C., Moreno, V., Herrero, R., Smith, J.S., Shah, K.V., Meijer, C.J.L.M., Bosch, F.X., for the International Agency for Research on Cancer (IARC) Multicentric Cervical Cancer Study Group (2002) Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet 2002, 359, 1093-101.

Muñoz, N., Bosch, F.X., de Sanjosé, S., Herrero, R., Castellsagué, X, Shah, K.V., Snijders, P.J.F., Meijer, C.J.L.M., for the International Agency for Research on Cancer Multicenter Cervical Cander Study Group (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer.
N. Eng. J. Med, 348(6): 518-27.

Muñoz, N., Bosch, F.X., Castellsagué, X., Diaz, M. de Sanjosé, S., Hammouda, D., Shah, K.V. & Meijer, C.J.L.M. (2004) Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int. J. Cancer, 111, 278–85.

Muñoz, N., Franco, E.L., Herrero, R., Andrus, J.K., de Quadros, C.A., Bosch, F.X. (2008) Recommendations for Cervical Cancer Prevention in Latin America and the Caribbean.
Vaccine. 2008 Aug 19;26 Suppl 11:L96-L107.

From causality to Prevention: The case of cervical cancer

Nubia Muñoz

Worldwide, cervical cancer is the second most common cancer in women, with about half of million cases being diagnosed every year and over 80% of these cases occurring in developing countries. It is the second most common cause of death from cancer among young women, accounting for nearly 300,000 deaths annually (1). Its main public health importance lies in the fact that it affects relatively young poor women, devastating their families and being an important cause of lost years of life in the developing world. This cancer reflects more than any other cancer the substantial inequities that exist in health. A major discovery in human cancer etiology has been the recognition that cervical cancer is a rare consequence of an infection by some mucosatropic types of Human Papillomavirus (HPV). In Public Health terms, the importance of this finding is comparable to the unveiling of the association between cigarette smoking and lung cancer, or between chronic infections with Hepatitis B or Hepatitis C viruses and the risk of liver cancer. This discovery has led to great advances in the prevention of this disease on two fronts: (i) primary prevention by the use of prophylactic HPV vaccines; and (ii) secondary prevention by increasing the accuracy of cervical cancer screening.

Although already 166 years ago Rigoni Stern thought that a sexually transmitted agent could be linked to cervical cancer, only during the last 25 years the human papillomavirus (HPV) has been identified as main cause of this cancer.

I have had the privilege of being one of the scientists that participated in this discovery. My first observation goes back to 1974 when I tried to link the high prevalence of giant condyloma with the high incidence of cancer of the cervix and of the penis in Recife, Brazil. In collaboration with Gerard Orth from the Pasteur Institute in Paris we looked for HPV particles in biopsies from giant condylomas, from cervical cancer and from cancer of the penis. Unfortunately, since HPV can not be grown in vitro, at that time, electron microscope was the only technology available to look for the virus in tissues. A few particles were seen in the condylomas but not in the genital cancers. Today we know that once the cancer is established, HPV viral particles are not longer present in the malignant cells, but fragments from its genes. In the same samples we looked for HSV-2 DNA in collaboration with Harald zur Hausen, with negative results. In the late 1970s Harald zur Hausen proposed that HPV may be one of the initiators of the carcinogenic process in the cervical epithelium (2). He, Lutz Gissmann and other scientist from his group, subsequently conducted groundbreaking research that led to the molecular characterization of HPV DNA isolated from cervical cancer samples (3-4). The demonstration of this series of molecular events was essential for the scientific community to accept that HPV infection was the likely cause of cervical cancer. Reasons for scepticism at the time came from observations in his own laboratory and in others that HPV infection was quite ubiquitous and, as such, it could not plausibly be a cause of disease, since a large proportion of asymptomatic women harboured HPV DNA in their cervices. In addition, formal epidemiological evidence of an association between HPV and cervical cancer was lacking at that time (5). HPV natural history studies have now revealed that HPVs are the commonest of the sexually transmitted infections in most populations. Most HPV exposures result in spontaneous clearance without clinical manifestations and only a small fraction of the infected persons, known as chronic or persistent carriers, will retain the virus and progress to precancer and cancer. Molecular characterization and cloning of the first HPV types in the early 1980s (3), made possible the development of hybridization assays to look for HPV gene fragments in human tissue.

Using PCR-based hybridization assays at my former Unit at the International Agency for Research on Cancer (IARC) we undertook the following epidemiological studies to investigate the role of HPV in cervical cancer:

Case- control studies

In 12 countries around the world we studied a total of 2,500 women with cervical cancer and 2,500 control women without cancer. These women were interviewed using a standardized questionnaire to elicit information on risk factors for cervical cancer and underwent a gynecological examination to collect cervical cells from the tumours and normal cervices for the detection of HPV DNA of 30 HPV types that infect the genital tract. The prevalence of HPV DNA was over 95% in the tumor cells of women with cervical cancer and it ranged from 5 to 20% in normal cervical cells of control women.

These prevalences correspond to Odds Ratios (ORs) of over 100 indicating a very strong association between HPV and cervical cancer. The magnitude of the ORs allowed an epidemiological classification of 15 HPV types as carcinogenic or high-risk types, 12 as low-risk types and 3 types as probably carcinogenic (6-7). This epidemiological classification correlates quite well with the phylogenetic classification based on sequencing of L1 gene.

Our case-control studies also allowed the identification of the following cofactors that acting together with HPV increase the risk of progression from HPV persistent infection to cervical cancer: tobacco, high parity, long term use of oral contraceptives and past infections with herpes simplex virus type 2 and Chlamydia trachomatis. (8-9). In addition, this studies contributed to establish the important role of male sexual behavior in the risk of developing cervical cancer (10-11).

Survey of HPV types in invasive cervical cancers

Over 1,000 women with invasive cervical cancer from 22 countries around the world were included in this study. HPV DNA detection with PCR-based assays revealed that 99.7% of the cases were HPV-positive. This finding led us to propose for the first time that HPV was not only the main cause of cervical cancer, but also a necessary cause (12). No other cancer has been shown to have a necessary cause.

The above two studies made possible the estimation of the proportion of cervical cancer cases attributable to the main HPV types in the various geographical regions. These estimates are being used to estimate the impact of preventive strategies based on HPV (13).

Implications

The demonstration that infection with certain types of human papillomavirus (HPV) is not only the main cause but also a necessary cause of cervical cancer has led to great advances in the prevention of this disease on two fronts:

(i) In primary prevention by the use of prophylactic HPV vaccines.

Two safe and efficacious prophylactic HPV vaccines have been developed using viral like particles (VLPs); the quadrivalent vaccine (Gardasil) contains VLPs of HPV 16 and 18, responsible for about 70% of cervical cancers, and VLPs of HPV6 and 11 that cause about 90% of genital warts. The bivalent vaccine (Cervarix) contains only VLPs of HPV16 and 18. Both vaccines have been shown to have a high efficacy for the prevention of high-grade precancerous lesions of the cervix (CIN2/3) and this protection has been shown to last at least 5 years (14-16). The quadrivalent vaccine has been shown in addition to prevent high-grade precancerous lesions of the vulva and vagina caused by HPV16 and 18 and genital warts caused by HPV 6 and 11 (14, 16). Universal vaccination of adolescent girls offers a great potential for the prevention of cervical cancer. Both vaccines have been licensed in over 100 countries but their high price limits their accessibility in the countries that need them most; it is hoped that a special price for developing countries could be negotiated with the pharmaceutics companies (17).

(ii) In secondary prevention by increasing the accuracy of cervical cancer screening.

Several studies have shown that HPV DNA detection assays are more sensitive than cytology for detection of high grade precursor lesions of the cervix (CIN2/3) and suggest that they should be used as primary screening test followed by triage with cytology or visual inspection (18). Evidence suggests that if the current HPV vaccines were introduced into developing countries and combined effectively with appropriate secondary cervical screening strategies, the lifetime risk of developing cervical cancer could be reduced as much as 60%. Mathematical models have shown that if the cost per vaccinated girl is less than $25, HPV16/18 vaccination would be very cost-effective in all 33 Latin American countries (19). The current price of the commercially available HPV tests is also the main barrier for their widespread introduction in developing countries. It is hoped that a fast and inexpensive HPV test developed with funds from the Gates foundation, will shortly be commercially available (20).

References

1. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide IARC CancerBase No. 5. version 2.0, IARCPress, Lyon, 2004.

2. zur Hausen H. Condylomata acuminate and human genital cancer. Cancer Res 1976 Feb;36(2 pt 2):794

3. Gissmann L zur Hausen H. Human papillomavirus DNA: physical characterization and genetic heterogeneity. Proc Natl Acad Sci USA 1976 Apr;73(4): 1310-3

4. Durst, M Gissmann L and zur Hausen H. A papillomavirus DNA from a cervical carcinoma and prevalence in cancer biopsy samples from diferent geographical regions. Proc Natl Acad Sci USA 1983 Jun80(12):3812-15

5. Muñoz N, Bosch X, Kaldor JM. Does human papillomavirus cause cervical cancer? The state of the epidemiological evidence. Br J Cancer. 1988 Jan;57(1):1-5.

6. Muñoz, N., Bosch, F.X., de Sanjosé, S., Tafur, L., Izarzugaza, et al. (1992) The causal link between human papillomavirus and invasive cervical cancer: a population based case control study in Colombia and Spain. Int. J. Cancer, 52, 743 49.

7. Muñoz, N., Bosch, F.X., de Sanjosé, S., Herrero, R., et al for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N. Eng. J. Med, 348(6): 518-27.

8. Muñoz, N., Franceschi, S., Bosetti, C., Moreno, V., Herrero,et al., for the International Agency for Research on Cancer (IARC) Multicentric Cervical Cancer Study Group (2002) Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet 2002, 359, 1093-101

9. Muñoz, N., Castellsagué, X., de Gonzalez, A.B., Gismann, L. (2006) Chapter 1: HPV in the etiology of human cancer. Vaccine, 24(3), 1-10.

10. Muñoz, N., Castellsagué, X., Bosch, F.X., Tafur, L., de Sanjosé, S., Aristizabal, N, Ghaffari, A.M. & Shah, K.V. (1996) Difficulty in elucidating the male role in Colombia, a high-risk area for cervical cancer. J. Natl Cancer Inst., 88, 1068-75.

11. Castellsagué, X., Bosch, F.X., Muñoz, N., Meijer, C.J.L.M., Shah, K.V., de Sanjosé, S., Eluf-Neto, J., Ngelangel, C., Chichareon, S., Smith, J.S., Herrero, R., Franceschi, S., for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. (2002) Male circumcision, penile human papillomavirus infection, and cervical cancer. New Engl. J. Med. 346(15): 1105-1112

12. Walboomers, J.M.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J.F., Peto, J., Meijer, C.J.L.M. & Muñoz, N. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J. Pathol., 189, 12-19.

13. Muñoz, N., Bosch, F.X., Castellsagué, X., Diaz, M. de Sanjosé, S., Hammouda, D., Shah, K.V. & Meijer, C.J.L.M. (2004) Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer, 111, 278–285

14. Ault KA; Future II Study Group. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma in situ: a combined analysis of four randomised clinical trials. Lancet. 2007 Jun 2;369(9576):1861-8.

15. Paavonen J, Jenkins D, Bosch FX, Naud P, Salmeron J, Wheeler CMet al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. Lancet 2007;369(9580):2161-70.

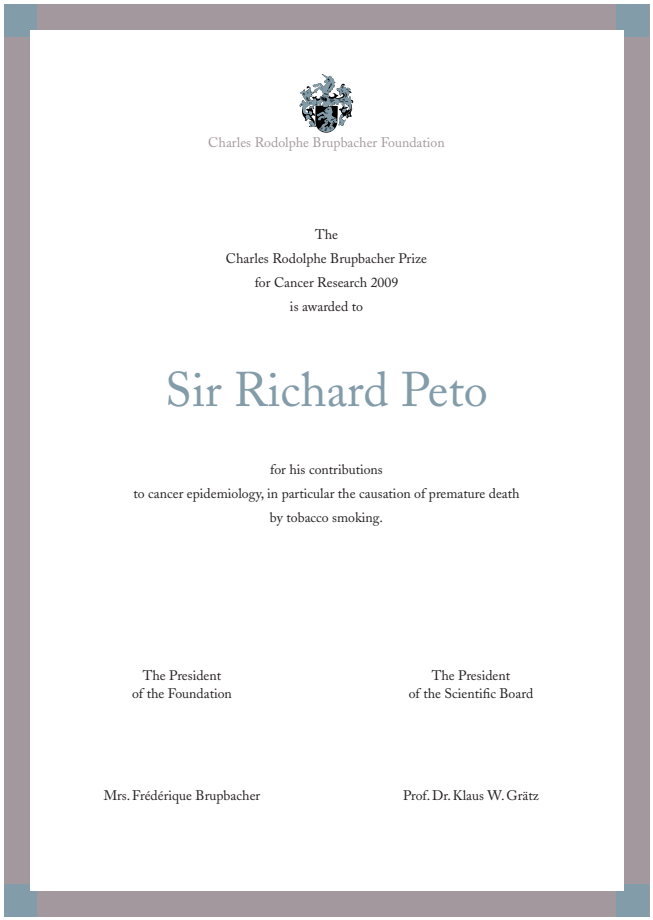
16. Villa LL, Costa RLR, Petta CA, Andrade RP, Paavonen J, Iversen O-E, et al. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. Br J Cancer 2006 Nov 21;95(11):1459-66.

17. Muñoz, N., Franco, E.L., Herrero, R., Andrus, J.K., de Quadros, C.A., Bosch, F.X. (2008) Recommendations for Cervical Cancer Prevention in Latin America and the Caribbean. Vaccine 2008;26(11), L96-L107

18. Cuzick J, Clavel C, Petry KU, Meijer CJLM, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer 2006;119:1095–105.

19. Goldie SJ, Diaz M, Constela D, Alvis N, Andrus JK, and Kim SY Mathematical models of cervical cancer prevention in Latin America and the Caribbean. Vaccine 2008;26(11), L59-L72

20. Qiao YL, Sellors J, Eder PS et al . Anew test for cervical cancer screening in developing regions : a cross-sectional study of clinical accuracy in rural China. Lancet Oncology Vol9 Oct 2008:929-36



Laudatio

Paul Kleihues

No human cancer is so tightly linked to a causal environmental factor as lung cancer is to smoking. Although this relationship is now universally accepted, it took a surprisingly long time to appreciate the magnitude of the adverse effects that smoking has on human health.

Today, we honour Sir Richard Peto, a scientist who has made groundbreaking studies on tobacco and cancer. His early studies with the late Sir Richard Doll showed a clear causal relationship, but the relative risk was not fully recognized. We now know that the risk of lung cancer in life-long smokers is more than 20 times that of non-smokers and that risk for cancer in many other organs is increased, including head and neck, urinary bladder, kidney, oesophagus and pancreas. It was the pioneering work of Doll and Peto that demonstrated the enormous burden of mortality associated with smoking. In the famous study on a cohort of more than 34 000 male British doctors who were monitored over five decades (1951-2001), it was revealed that the life expectancy of persistent cigarette smokers is markedly reduced. Men born in 1900-1930 who smoked only cigarettes and continued smoking died on average 10 years younger than lifelong non-smokers. On the positive side, this work also showed that cessation of smoking significantly reduces the risk of lung cancer even after extensive periods of smoking. Stopping smoking at age 30 largely diminished the adverse effect on life expectancy and cessation as late as at age 50 gained about 6 years of life expectancy and reduced the risk of dying of lung cancer before age 75 by more than 50%. An important conclusion from this study is that public health action should not only concentrate on discouraging young people from taking up the smoking habit, but that equal emphasis should be placed on persuading present smokers to quit.

Importantly Sir Richard Peto has adopted a global approach to epidemiology, extending his research on smoking to other world regions, and in particular to India and China. Peto has estimated that in the 20th century worldwide more than 100 million people died prematurely from smoking and that the high prevalence

of smoking in populous Asian countries will ultimately cause a much higher death toll in the 21st century.

Local opinion in some developing countries had favoured the view that smoking- related disease is an exclusive problem of western nations. Professor Peto demonstrated that after a typical lag period, the same disease burden could be seen in all communities, largely independent of ethnicity and lifestyle factors other than smoking. A large study in India revealed that smoking was associated with a reduction in survival of 8 years for women and 6 years for men and that in 2010, smoking will cause about 930,000 adult deaths in India. Of these, about 70% will occur between the ages of 30 and 69 years.

Through the weight of his studies and as outspoken critic, Richard Peto was and probably still is, the nemesis of the tobacco industry. However, times have changed. Even tobacco producers now have to admit that they sell a deadly product and their public denial of nicotine addiction has further weakened their credibility.

Sir Richard Peto has been awarded multiple honours, many of which reflect his particular contributions to health and medical research generally. Fellowship of the Royal Society was accorded for introducing epidemiological meta-analyses. From such meta-analyses he demonstrated not only the life-saving effects of tamoxifen for breast cancer treatment but also the life-saving effects of many treatments for heart disease. He received the European Award for Excellence in Stroke Research.

Notwithstanding these and many other contributions, however, his work on smoking has been his greatest life-saving achievement. Sir Richard's work has clearly shown that tobacco abuse is world-wide by far the most important avoidable cancer risk. His empathic presentation of these findings has been a key factor in persuading governments to adopt anti-smoking policies to extend the lifespan of smokers able to quit and to protect non-smokers from starting.

Sir Richard Peto

Curicculum vitae and Publications

Date of birth	14 May 1943
Home address	University of Oxford Nuffield Departement of Clinical Medicine CTSU Richard Doll Building Old Road Campus Roosevelt Drive Oxford, OX3 7LF UK
Current position	Professor of Medical Statistics & Epidemiology, University of Oxford; Co-director, with Professor Rory Collins, of the University's Clinical Trial Service Unit & Epidemiological Studies Unit (CTSU)

Education

1965	BA Natural Sciences, University of Cambridge
1967	MSc Statistics (with distinction), University of London

1974	MA, University of Cambridge (& hence, by incorporation, of Oxford)

Appointments

1967 - 1969	Research Officer, Medical Research Council Statistical Research Unit, London
1969 -1979	Research Officer (1969-72), Lecturer (1972-75) & Reader (1975-79), Department of the Regius Professor of Medicine, University of Oxford
1979 - 1992	University Reader in Cancer Studies, Nuffield Department of Clinical Medicine, University of Oxford
1985- to date	Co-director, Clinical Trial Service Unit & Epidemiological Studies Unit (CTSU)
1992- to date	Professor of Medical Statistics and Epidemiology, Nuffield Department of Clinical Medicine, University of Oxford

Honorary Appointments

1979- to date	Foundation Fellow, Green College, University of Oxford
1989- to date	Honorary Professor, Chinese Academy of Preventive Medicine, PRC
1990- to date	Adjunct Professor, Cornell University, USA
1995- to date	Honorary Professor, Peking Union Medical College, PRC
2006- to date	Honorary Fellow, London School of Hygiene & Tropical Medicine

Honours & Awards

1986	Guy Silver Medal, Royal Statistical Society, for development of the logrank test
1987	Honorary Membership of the Royal College of Physicians of London
1988	Honorary Member, Swedish Society of Internal Medicine
1989	Fellowship of The Royal Society of London (FRS), for development of meta-analyses of trial results
1989	Helmut Horten Foundation Award (with Sir Richard Doll)
1989	Honorary Professor, Chinese Academy of Preventive Medicine (now CDC), for demonstrating the hazards of tobacco in China
1990	Adjunct Professor, Cornell University, USA
1992	Honorary Fellow, Faculty of Public Health Medicine
1992	Honorary Doctorate, University of Tampere, Finland
1992	General Motors Visiting Professor, IARC, Lyon, France
1992	Gairdner Foundation Award, Canada
1992	Caradog Jones Lecturer 1992, Royal Statistical Society
1993	Frohlich Award of the New York Academy of Sciences
1993	Donald Reid Medal, London School of Hygiene
1993	Polish Cardiac Society Medal of Merit
1994	La Médaille de la Ville de Paris (Échelon Vermeil)
1995	European Award for Excellence in Stroke Research
1996	Oettlé Memorial Medal, South Africa
1996	Prix Raymond Bourgine for Achievement in Cancer Research
1997	Gold Award, Polish Health Promotion Foundation
1997	Prix Louis Jeantet for Medicine
1997	10th World Conference on Tobacco or Health Award
1998	Founder Fellow, The Academy of Medical Sciences
1998	Fothergill Medal of the Medical Society of London (with Rory Collins)
1998	Leverhulme Prize, Liverpool School of Tropical Medicine (with Alan Lopez)

1999	Honorary Fellowship of the Royal College of Physicians of London
1999	Rickman Godlee Lecturer, University College, University of London
1999	Hon DSc, University of London
1999	Knighthood awarded in the Queen's birthday honours list, for services to epidemiology and to cancer prevention
2000	Prince Mahidol Award for Public Health, Bangkok, Thailand (with I Chalmers)
2000	Polish Presidential Public Health Award
2000	International Aspirin Senior Award
2001	Academician, Academy of Finland
2001	Lynn Sage Distinguished Award in Breast Cancer Research, for the Early Breast Cancer Trialists' Collaborative Group
2002	Order of Merit of the Republic of Poland (Officer Cross)
2002	Membre Étranger Associé de l'Académie des Sciences (France)
2002	Norwegian Cancer Society: King Olav V prize, for outstanding cancer research (with R. Doll)
2002	General Motors Cancer Research Foundation: Mott prize, for cancer prevention
2002	The Royal Society: The Royal Medal, for studies of smoking and chronic disease
2003	Honorary DSc, University of Southampton
2003	Distinguished Service Award, American Society for Clinical Oncology (ASCO), for scientific leadership
2004	Weldon Memorial Medal, University of Oxford
2004	Visiting professor, Johns Hopkins School of Medicine
2004	Gold medal of the Royal Society for the Promotion of Health
2004	Honorary Fellow of the RCP Faculty of Pharmaceutical Medicine, London
2004	International Lecture on Prevention, German Cardiac Society
2004	Elected to Foreign Associate Membership, US Institute of Medicine
2005	King Faisal International Prize for Medicine (jointly with R Doll)
2005	Chair, WHO high-level scientific advisory panel on health statistics
2005	Federation of European Cancer Societies (FECS) Annual Clinical Research Award
2005	European Lung Foundation (ELF) Annual Award, for service to human health
2005	Cutter Preventive Medicine Lectureship, Harvard School of Public Health (www.hsph.harvard.edu/cutterlecture)
2006	Honorary Fellow, London School of Hygiene, for epidemiology and prevention of non-communicable diseases
2006	American Cancer Society Luther L Terry Award, for outstanding research contributions to tobacco control
2006	Queen's Anniversary Prize for Higher and Further Education, 2006-2010, awarded to the University of Oxford for the research studies of the CTSU
2007	MacKenzie Medal, British Cardiovascular Society, for outstanding service to cardiology
2008	WHO International Agency for Research on Cancer Medal of Honour, for outstanding contribution to cancer research.
2008	Heineken Prize, Royal Netherlands Academy of Arts and Sciences

The absolute benefits of anti-cancer drugs and of tobacco control

Sir Richard Peto

Cancer treatment and cancer prevention are both important. Even 50 years ago, before there were effective anti-cancer drugs, many people with some of the common types of cancer were completely cured by successful surgery. Nowadays, helped by screening and early detection, cancer surgery and radiotherapy still save more lives than cancer drugs do. The first anti-cancer drugs were, unfortunately, effective only against uncommon forms of the disease, such as the cancers of childhood or of early adult life. So, although for the few young people who actually had cancer the benefit from those old chemotherapy regimens could be large (because their risk of death was halved), for the population as a whole the absolute gain was relatively small. Modern drug treatments for the cancers of childhood and early adult life have reduced the population risk of dying from cancer before age 35 from 0.4% in the 1950s to 0.2% today, but this is an absolute gain of only 0.2%.

Only 25 years ago there was still, in many countries, a widespread nihilistic belief among many doctors about the drugs then available for the common types of cancer that although medical treatment could shrink such a tumour temporarily it couldn't ever cure the patient. Indeed, in the early 1980s many of the doctors who collaborated in randomised trials of drugs for common diseases like breast cancer or intestine cancer expected merely to help demonstrate that in the long run treatment with nasty anti-cancer drugs did not cure anybody, thereby at least protecting future patients from inappropriate over-treatment by over-zealous colleagues.

The trouble was, however, that back in the 1980s all of the randomised trials of the treatment of common cancers were too small to be statistically reliable on their own. So, in the mid-1980s we in Oxford got all of the breast cancer trialists in the world to collaborate and share their data with us. To our surprise and theirs, when the results from many different trials that had addressed much the same therapeutic question in breast cancer were added together, we did see some small but definite effects on five-year survival — hormonal therapy did something small but real, and so did chemotherapy.

Nobody really knew whether these small survival differences would be transient or permanent, so the trialists agreed that they would all share their data again every 5 years, in 1990, 1995, 2000, 2005 and so on; they've continued to do so, and they'll do it again next year, in 2010. The good news was that the small gains in 5-year survival did not disappear. Indeed, long follow-up showed that the differences in 10-year and in 15-year survival from hormonal therapy, from chemotherapy and from radiotherapy were slightly bigger than the differences in 5-year survival had been, and although the 10-year benefits still weren't very big, hormone therapy and chemotherapy can both be given, adding the two benefits together. Moreover, continuation of the worldwide collaboration between many trials over many years showed that some of the newer types of chemotherapy and hormonal therapy were slightly better than the older ones, and eventually, by a series of small but definite steps forward over the past 25 years, we've got to the point where about half the women who would have died of their breast cancer will not now do so, because of earlier diagnosis and better treatment. Hence, even though more women develop breast cancer nowadays, the national breast cancer mortality rates in many countries are definitely falling, and I hope they'll keep on doing so. It's still not a very big absolute gain, but it is real. In the UK female population, for example, the probability of death before age 70 from breast cancer has gone down over the past 20 years from about 2.5% to 1.5%, which is an absolute gain of 1%. Not good enough, but not bad.

Control of the main cause of cancer offers considerably greater absolute benefits, however, and in Europe and North America much the biggest cause of cancer is tobacco. Smoking is more important than all other known causes of cancer added together, and it causes even more deaths from other diseases than from cancer.

When we compared men in Britain who had smoked cigarettes throughout adult life with men who had never smoked, we found a 10-year difference in life expectancy. (Men who had

stopped at age 30 did almost as well as the never-smokers.) That 10-year difference is about twice as big as other studies had suggested, because ours was the first clean study of men who had smoked substantial numbers of cigarettes throughout adult life. Partly as a result of our study, two-thirds of the smokers my age in Britain have now stopped, lung cancer rates are falling, and, as smoking kills even more people by other diseases than by lung cancer, the overall death rates from smoking are falling substantially, particularly in men. The proportion of UK males killed by smoking at ages 35-69 has already decreased from 20% in 1970 to 5% today, which is an absolute population gain of 15%, and it's still falling. British men have had the best decrease in tobacco deaths in the world (partly because they used to have the worst death rates from tobacco in the world), but there are also substantial decreases in tobacco deaths in several other developed countries, though not in all.

The really bad news today comes from big developing countries like China and India. We have worked with Chinese scientists in the 1980s and with Indian scientists in the present decade on large nationwide studies of tobacco deaths. Both in China and in India there are already about one million tobacco deaths a year, the annual number is rising, and tobacco consumption isn't falling.

Worldwide, there were about 100 million deaths from tobacco during the 20th century and there will be about 1000 million this century, if current smoking patterns continue (with widespread uptake of smoking and, at least in developing countries, little cessation until people are already ill). Practicable changes in public policy could avoid tens of millions of premature deaths over the next few decades, and could avoid hundreds of millions over the whole century. For example, the French government recently tripled the price of cigarettes, French cigarette consumption fell by half, and the government got richer. It's a much easier way to save lots of lives than improving cancer treatment is, but fortunately we can do both.

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»

Programm des Wissenschaftlichen Symposiums 2009

Program of the Scientific Symposium 2009

Wednesday, February 11, 2009

13:00 - 14:30 *Registration / Coffee*

14:30 - 16:00 **Signal Transduction Pathways as Targets for Cancer Prevention and Therapy**
Chair: Holger Moch

Moshe Oren, Rehovot
P53 and cancer: One gene, many faces

Klas G. Wiman, Stockholm
Restoration of mutant p53 function as therapeutic strategy

Webster K. Cavenee, La Jolla
Targeted therapies: The importance of signaling molecules

16:00 - 16:30 *Coffee break*

16:30 - 17:30 **Gene regulation**
Chair: Susan Gasser

Christoph Plass, Heidelberg
DNA promoter methylation in cancer

Joseph Nevins, Durham
Genomic strategies for personalized cancer therapy

17:30 - 18:30 *Apéro*

19:00 - 20:00 **Charles Rodolphe Brupbacher Lecture**

Thomas Zeltner, Bern
Krebsprävention als politische Herausforderung

Thursday, February 12, 2009

08:00 - 08:30 *Registration*

08:30 - 10:00 **Cancer Causes and Prevention (1)**
Chair: Chris Wild

D. Maxwell Parkin, Oxford
The global burden of cancer: Trends and perspectives

Nubia Muñoz, Lyon
From causality to prevention: The case of cervical cancer

Curtis C. Harris, Bethesda
MicroRNAs and inflammatory cytokines as biomarkers of cancer diagnosis, prognosis, and therapeutic outcome

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

11:00 - 12:00 **Cancer Causes and Prevention (2)**
Chair: Pierre Clavien

Rudolf Kaaks, Heidelberg
Nutritional energy balance and cancer risk: Epidemiological evidence implicating insulin and insulin-like growth factor-I

Sir Richard Peto, Oxford
Changing cancer mortality

12:00 - 13:00 *Lunch + Posters No 1 - 60*

13:00 - 14:00 *Lunch + Posters No 61 - 118*

14:00 - 14:30 *Coffee break*

14:30 - 16:30 **Cancer Genetics, Gene Function and Metastasis**
Chair: Josef Jiricny

Olli P. Kallioniemi, Helsinki
Diagnostic and therapeutic discoveries from genome-scale functional and translational cancer research

Carolyn C. Compton, Bethesda
The Cancer Genome Atlas (TCGA) Project

William G. Kaelin, Boston
The von Hippel-Lindau tumor suppressor gene: Oxygen sensing and cancer

Michael Karin, San Diego
How metastatic cells usurp innate immunity and inflammatory processes

16:30 - 17:00 *Break*

17:00 - 18:30 **Award Ceremony and Lectures of the Awardees**

18:30 - 19:30 *Apéro*

Friday, February 13, 2009

08:30 - 10:00 **Apoptosis and DNA Damage**
Chair: Michael Arand

Simone Fulda, Ulm
Apoptosis proteins as targets for anticancer therapy

Ulrich Hübscher, Zürich
Repair of oxydative damage

Carlo Croce, Columbus
micro-RNA mediated suppression of tumorigenesis

10:00 - 10:30 *Coffee break*

10:30 - 12:00 **Stem Cells**
Chair: Alexander Knuth

Lukas Sommer, Zürich
Cancer stem cells in melanoma: Potential culprits for tumor growth and metastasis

Jörg Huelsken, Lausanne
Cutaneous cancer stem cells

Michael F. Clarke, Stanford
Molecular analysis of normal and malignant epithelial stem cells

12:00 - 12:30 **Frédérique Brupbacher, Holger Moch**
Presentation of Charles Rodolphe Brupbacher Young Investigator Awards

Paul Kleihues
Closing Remarks

Abstracts
Eingeladene Redner

Abstracts
Invited Speakers

Krebsprävention als politische Herausforderung

Prof. Thomas Zeltner

Direktor Bundesamt für Gesundheit

Krebs ist die zweithäufigste Todesursache in der Schweiz. Im Laufe des Lebens erkranken vier von zehn Menschen der Schweiz an Krebs, über 30'000 Krebsfälle werden pro Jahr neu diagnostiziert. Auch wenn der wichtigste bekannte Risikofaktor für viele Krebsarten das Alter ist, könnte gemäss der WHO mindestens ein Drittel der auftretenden Krebserkrankungen vermieden werden. Das Rauchen und das Ernährungs- und Bewegungsverhalten (zunehmendes Übergewicht) sind die wichtigsten potentiell modifizierbaren Risikofaktoren. Die demografische Entwicklung und die zunehmende Verbreitung von Übergewicht und Adipositas in der Schweiz lassen in den nächsten Jahren und Jahrzehnten eine Zunahme der Krebsfälle erwarten. Eine Verbesserung des Gesundheitszustandes der Bevölkerung durch Prävention und Gesundheitsförderung ist deshalb kein Luxus, sondern eine Notwendigkeit.
Dabei ist zu berücksichtigen, dass sich auch sozioökonomische Faktoren, das Geschlecht oder die ethnische Herkunft auf das Gesundheitsverhalten und damit die Gesundheit auswirken. So leben etwa Menschen mit einer geringeren Schulbildung weniger lange als jene, die höher gebildet sind. Will man den Gesundheitszustand des Einzelnen verbessern, muss man auch seine Lebens- und Arbeitsbedingungen mit einbeziehen.
Krebsprävention ist also ein weites Feld: Eine programmatische Verhütung von Krebs geht von Primärprävention, d.h. Verhinderung der Krebsentstehung durch präventive und gesundheitsfördernde Massnahmen (etwa in den Bereichen Tabakprävention, Ernährungsverhalten, Bewegung), über Sekundärprävention (Früherkennungsmassnahmen) bis zu tertiärer Prävention (Verhütung der Verschlimmerung von Erkrankungen, Vorbeugung von Folgeerkrankungen). Weitere wichtige Pfeiler der Krebsprävention sind die medizinische Prävention (Impfungen), die Förderung der Gesundheitskompetenz (Health Literacy), die Forschung und die epidemiologische Überwachung (Krebsregister).
Handlungsbedarf besteht zudem auf gesetzlicher Ebene. Einerseits mangelt es an einer Gesamtstrategie im Sinne übergeordneter Präventions- und Gesundheitsförderungsziele, andererseits fehlen dem Bund die gesetzlichen Grundlagen, um in der Prävention von nichtübertragbaren Krankheiten aktiv zu werden. Mit der Schaffung eines nationalen Präventionsgesetzes sollen diese Lücken geschlossen und eine solide Basis für die zukünftige Ausgestaltung von Prävention und Gesundheitsförderung in der Schweiz gelegt werden.

P53 and cancer: One gene, many faces

Neta Moskovits, Jair Bar, Perry Stambolsky, Varda Rotter and Moshe Oren

Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

p53 is a pivotal tumor suppressor (1). The TP53 gene, encoding p53, is mutated in about half of all human tumors, making it perhaps the most frequently altered gene in human cancer. The centrality of p53 as a tumor suppressor is probably due, at least in part, to the fact that it can monitor and respond to a wide variety of stress signals, all of which are associated in one way or another with tumorigenesis. The p53 protein is a transcriptional regulator. In response to such oncogenic stress signals, p53 is activated and modulates the expression of a multitude of target genes. Such transcriptional modulation elicits extensive changes in cell fate, including apoptosis and replicative senescence, and serves to abort the propagation of neoplastically-transformed cells. Loss of normal p53 function thus opens the gate to tumor progression.
The role of p53 has been extensively studied within tumor cells and cells that are at risk of becoming tumorous. However, recent studies indicate that p53 can exert its tumor suppressor activity also within the stromal compartment of the tumor. For instance, p53-deficient fibroblasts can preferentially augment the growth of prostate cancer cell-derived tumors in a xenograft model. Thus, in addition to acting in a cell-autonomous manner to suppress malignant transformation, p53 also possesses a non cell-autonomous tumor suppressor activity in the stroma. Of note, p53-deficient fibroblasts produce elevated levels of secreted proteins such as SDF-1/CXCL12, which may facilitate tumor growth and spread (2). Furthermore, the p53 status of human fibroblasts affects their response to signals from neighboring tumor cells, as measured by alterations in gene expression patterns in such fibroblasts when exposed to conditioned medium of cultured human cancer-derived cells. Quenching p53 function in stromal fibroblasts may therefore provide tumor cells with a selective advantage by enabling them to evade the potential non cell-autonomous tumor suppressor activity of p53. Indeed, we found that epithelial tumor cells can repress p53 activation in both mouse and human fibroblasts. This ability is acquired when epithelial cells undergo neoplastic transformation. Interestingly, this p53-repressive effect of tumor cells is exerted more readily in cancer-associated fibroblasts (CAFs) than in normal fibroblasts.
While the wild type (wt) p53 protein is a potent tumor suppressor, there is growing evidence that cancer-associated mutant forms of p53 actually promote tumorigenesis and increase tumor aggressiveness, consistent with acquisition of an oncogenic gain of function (3). For instance, such mutant

p53 isoforms can exert anti-apoptotic effects; ablation of the endogenous mutant p53 can render tumor-derived cells more responsive to killing by a variety of anti-cancer agents. Interestingly, chromatin immunoprecipitation (ChIP) analysis suggested that mutant p53 is preferentially associated with the chromatin of vitamin D-responsive genes. Further exploration of this finding revealed that mutant p53 can interact with the vitamin D receptor and modulate the transcriptional response to vitamin D. Notably, whereas vitamin D often induces apoptosis in tumor cells that retain wt p53 expression, the acquisition of p53 mutations can sometimes reverse the effect of vitamin D, rendering it cytoprotective. Hence, p53 mutations may alter profoundly the biological response of tumor cells to external signals, in a way that might augment tumor malignancy and compromise the efficacy of anti-cancer therapy.

1. Vousden, K. H., and Lane, D. P. (2007). p53 in health and disease. Nat Rev Mol Cell Biol 8, 275-283

2. Moskovits, N., Kalinkovich, A., Bar, J., Lapidot, T., and Oren, M. (2006). p53 Attenuates cancer cell migration and invasion through repression of SDF-1/CXCL12 expression in stromal fibroblasts. Cancer Res 66, 10671-10676.

3. Weisz, L., Oren, M., and Rotter, V. (2007). Transcription regulation by mutant p53. Oncogene 26, 2202-2211.

Restoration of mutant p53 function as therapeutic strategy

Klas G. Wiman

Cancer Center Karolinska (CCK), Karolinska Institute

The p53 tumor suppressor gene is mutated in a large fraction of human tumors. p53 mutation allows evasion from p53-dependent apoptosis upon cellular stress such as oncogene activation and aberrant DNA replication. Restoration of wild type p53 expression has been shown to trigger cell death and rapid elimination of tumors in vivo. The identification of mutant p53-targeting small molecules such as PRIMA-1 opens possibilities for the development of more efficient anticancer drugs. PRIMA-1 restores wild type conformation to mutant p53, induces apoptosis in human tumor cells, and inhibits xenograft tumor growth in vivo (1). We also showed that PRIMA-1 synergizes with chemotherapeutic drugs, both in cultured tumor cells and in vivo (2). PRIMA-1 induces the pro-apoptotic p53 target genes Bax and PUMA and activation of caspase-2, leading to loss of mitochondrial membrane potential, cytochrome c release, and activation of downstream effector caspases (3). Microarray analysis revealed that PRIMA-1 induces changes in expression of a limited number of genes in mutant p53-expressing cells. These include genes that regulate apoptosis as well as genes associated with cell cycle control and senescence. Experiments with radiolabelled PRIMA-1 indicated direct binding to mutant p53 in living cells. A better understanding of the cellular events triggered by PRIMA-1 treatment should facilitate the design of more potent and specific mutant p53-targeting anticancer drugs.

1. Bykov, V.J., Issaeva, N., Shilov, A., Hultcrantz, M., Pugacheva, E., Chumakov, P., Bergman, J., Wiman, K.G., & Selivanova, G. (2002) Restoration of the tumor suppressor function to mutant p53 by a low molecular weight compound. Nature Med. 8, 282-8.

2. Bykov, V.J.N., Zache, N., Stridh, H., Westman, J., Bergman, J., Selivanova, G. and Wiman, K.G. (2005) PRIMA-1MET synergizes with cisplatin to induce tumor cell apoptosis. Oncogene 24, 3484-91.

3. Shen, J., Vakifahmetoglu, H., Stridh, H., Zhivotovsky, B., and Wiman, K.G. (2008) PRIMA-1MET induces mitochondrial apoptosis via activation of caspase-2. Oncogene 27, 6571-80.

Targeted therapies: The importance of signaling molecules

Webster K. Cavenee, Ph.D.

Director Ludwig Institute for Cancer Research- San Diego Branch

Advances in understanding the genetic basis of cancer have led to the identification of molecules that are specifically expressed in tumors. Such molecules represent targets for therapy whose targeting might be expected to have increased selectivity for tumor cells compared to normal tissue with resultant enhancements in effect and reductions in the non-specific toxicities that lead to chemotherapeutic side effects. Two such molecules are 1) a mutated form of the epidermal growth factor receptor (EGFR); and, 2) the dual specificity phosphatase, PTEN. Both have been found in sizable proportions of tumors of the brain, lung, prostate and breast.

Several questions arise that could influence the selection and application of targeted therapies. First, what is the mode of action of these molecules and how do tumor-derived mutations affect these activities? We have determined the mode of action of both the mutant receptor and the phosphatase and demonstrated their role in tumor growth. Second, are these mutations required for the maintenance of the tumor or only for tumor initiation? We have shown the requirement of the mutated receptor for the maintenance of rapid tumor growth in vivo. Third, can specific therapeutics be developed that are directed at these oncogenic mutations? We have targeted the mutant receptor using small molecule inhibitors and specifically directed monoclonal antibodies. In preclinical studies, such approaches have had significant and remarkable effects on inhibition of tumor growth and extension of host lifespan in the absence of generalized toxicity. Third, do combinations of mutations in tumors determine responsiveness to particular therapies? In a large multigroup collaboration we have shown that patients with tumors that express mutant EGFRs respond to certain tyrosine kinase inhibitors only if their PTEN gene is wild type. Data in support for each of these assertions will be presented.

These results suggest the use of specifically and rationally targeted compounds in combinations for effective responses and as biomarkers for the stratification of patient groups.

1. Louis DN and Cavenee WK (2005). Molecular Biology of Central Nervous System Tumors. In Cancer: Principles and Practice of Oncology, VT DeVita, S Hellman and SA Rosenberg, eds. Lippincott-Raven Publishers, Philadelphia, pp. 1827-1834, 7th Edition.

2. Cavenee WK, Furnari FB, Nagane M, Huang H-JS, Newcomb EW, Bigner DD, Weller M, Berens M, Plate K, Israel M, Noble M and Kleihues P (2000). Diffusely Infiltrating Astrocytomas. In: World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Nervous System. 2nd Edition. P. Kleihues and W. Cavenee, eds. IARC Press, Lyon, France, pp 10-21.

3. Goudar RK, Shi Q, Hjelmeland MD, Keir ST, McLendon RE, Wikstrand CJ, Reese ED, Conrad CA, Traxler P, Lane HA, Reardon DA, Cavenee WK, Wang X-F, Bigner DD, Friedman HS and Rich JN (2005). Combination Therapy of Inhibitors of Epidermal Growth Factor Receptor/Vascular Endothelial Growth Factor Receptor 2 (AEE788) and the Mammalian Target of Rapamycin (RAD001) Offers Improved Glioblastoma Tumor Growth Inhibition. Molecular Cancer Therapeutics 4: 101-112.

4. Perera RM, Narita Y, Furnari FB, Gan HK, Murone C, Ahlkvist M, Luwor RB, Burgess AW, Stockert E, Jungbluth AA, Old LJ, Cavenee WK, Scott AM and Johns TG (2005). Treatment of Human Tumor Xenografts with mAB 806 in Combination with a Protypical EGFR-Specific Antibody Generates Enhanced Anti-Tumor Activity. Clinical Cancer Research 11: 6390-6399.

5. Mellinghoff I, Wang Y, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JHY, Chute DJ, Riggs BL, Horvath S, Liau LM, Cavenee WK, Rao PN, Beroukhim R, Peck TC, Lee JC, Sellers WR, Stokoe D, Prados M, Cloughesy TF, Sawyers CL and Mischel PS (2005). Molecular Determinants of the Response of Glioblastomas to EGFR Kinase Inhibitors. New England Journal of Medicine 353: 2012-2024.

DNA promoter methylation in cancer

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Two general mechanisms have been identified that are involved in the silencing of cancer related genes. Genetic alterations, including mutations and deletions, have been known to be involved in tumor suppression for many years. More recently, DNA methylation has been identified as an additional mechanism to silence genes (1). Aberrant DNA methylation is an early event in tumorigenesis and a major contributor in the development of solid tumors as well as leukemias. As an epigenetic alteration, DNA methylation does not change the sequence of a gene and thus offers the exciting possibility for therapeutic removal of the methylation group by demethylating drugs.

Deregulation of mechanisms that control the establishment of normal DNA methylation patterns leads to both extensive aberrant hypo- and hypermethylation and has been described for several human malignancies (2). Global DNA hypomethylation in human cancers was one of the earliest changes associated with tumor progression. Our group has shown that human malignancies are characterized by extensive promoter CpG island methylation with non-random and tumor-type specific patterns. It is currently unknown how tumors acquire aberrant DNA methylation patterns (3).

In this symposium our current understanding of epigenetic alterations will be discussed using the example of chronic lymphocytic leukemia (CLL). Data will be presented that describes the changes occurring in the epigenetic states in human CLL genomes (4, 5). Furthermore, we will present data on a mouse model that recapitulates epigenetic alterations and develops CLL.

1. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128(4):683-92.

2. Costello JF, Plass C. Methylation matters. J Med Genet 2001;38(5):285-303.

3. Costello JF, Fruhwald MC, Smiraglia DJ, et al. Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. Nature Genet 2000;24(2):132-8.

4. Rush LJ, Raval A, Funchain P, et al. Epigenetic profiling in chronic lymphocytic leukemia reveals novel methylation targets. Cancer Res 2004;64(7):2424-33.

5. Raval A, Tanner SM, Byrd JC, et al. Downregulation of death-associated protein kinase 1 (DAPK1) in chronic lymphocytic leukemia. Cell 2007;129(5):879-90.

Genomic strategies for personalized cancer therapy

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The ability to tailor cancer therapy to characteristics of the individual patient is key in achieving a successful outcome. We have made use of genomic data to develop predictors that assess risk and sensitivity to potential therapeutic options. The current standard of care for the majority of Stage 1 patients is surgery and observation. Nevertheless, a substantial fraction of these patients will have a disease recurrence suggesting the need to identify individuals in this subgroup for more effective therapy. We developed gene expression profiles that could predict risk of recurrence, performing significantly better than previously described clinical prognostic factors (1). Importantly, we show that this provides an opportunity to re-classify Stage 1 patients to identify a subset of higher risk patients that might be appropriate for adjuvant chemotherapy. This can be coupled with gene expression signatures that have the capacity to predict sensitivity to cytotoxic chemotherapeutics, including those used in the treatment of lung cancer, to guide the most effective chemotherapy for these patients (2). This concept is being tested in further prospective trials. Finally, the capacity to direct the use of new investigational drugs, particularly those that target oncogenic signaling activities, is critical to allow more efficient use of these agents and to develop strategies for rational combinational therapy. We have made use of expression signatures that predict pathway deregulation in cancer cell lines as a basis to guide the use of therapeutic agents that target components of the pathway (3). We have extended this concept to develop more refined signatures that can dissect the complexities of many of the known signaling pathways, providing a more precise capacity to probe the activity or deregulation of the pathway. Finally, recognizing the need to substantially increase the number of drugs available for these cancer patients, we have developed a novel approach that makes use of a phenotype-based screen combined with the use of multiple cancer cell lines. Targets are cancer-relevant phenotypes represented as gene expression signatures, including pathway signatures. These signatures are used to identify cancer cell lines reflecting the signature phenotype and then connect to compounds that are selectively active against those cells. This approach, when combined with the capacity to predict chemotherapy use, has the potential to identify therapeutic strategies that make use of all available drugs, matched to the characteristics of the individual patient and thus an approach towards the development of personalized treatment options for the individual patient.

1. Potti, A., Mukherjee, S., Prince, R., Dressman, H. K., Bild, A., Koontz, J., Kratzke, R. A., Watson, M., Kelley, M., Ginsburg, G. S., Harpole, D., and Nevins, J. R. A genomic strategy to refine prognosis in non-small cell lung carcinoma. N. Engl. J. Med. 2006; 355: 570-580.

2. Potti, A., Lancaster, J. M., Dressman, H. K., Bild, A., Riedel, R. F., Kelley, M., Berchuck, A., Petersen, R., Harpole, D., Ginsburg, G. S., Nevins, J. R., and Febbo, P. G. A genomic strategy to guide the use of chemotherapeutic drugs in solid tumors. Nature Medicine 2006; 12: 1294-1300.

3. Bild, A., Yao, G., Chang, J. T., Wang, Q., Potti, A., Chasse, D., Joshi, M.-B., Harpole, D., Lancaster, J. M., Berchuck, A., Olson, J. A., Marks, J. R., Dressman, H. K., West, M., and Nevins, J. R. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature 2006; 439: 353-357.

The global burden of cancer: Trends and perspectives

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At the world level, as in a local Public Health department, it is essential to know the dimensions of the health problems, in order to prioritise resources (research, prevention, treatment), and to monitor the effect of interventions. For cancer, the International Agency for Research on Cancer (IARC) has been preparing worldwide estimates since 1984, and the latest, estimating number of cases, deaths and prevalent cancers (GLOBOCAN 2002) have just been prepared. The data sources, and methods, have slowly improved over time. There are two major data sources: national mortality statistics, and incidence and survival from the worldwide network of cancer registries. Mortality data may be useable directly, but may need some manipulation to correct for incomplete registration, or non-specific coding. Cancer registry data may be available nationally, but often national estimates have to be prepared using incomplete (regional) data. Mortality can be estimated from incidence, and vice versa, using survival; prevalence is estimated from incidence and survival.

We believe that there were 10.8 million new cases, 6.7 million deaths and 22 million persons alive with cancer in the year 2002. Now, 54% of cases occur in the so-called developing countries. The most common cancers are lung (1.35 million), breast (1.15 million), colon-rectum (1.02 million), stomach (930,000), prostate (679,000), liver (623,000) and cervix (493,000), but the ranking varies enormously by world region. For example, the most common cancers of men in sub-Saharan Africa is now Kaposi sarcoma (and liver cancer in second).

Because of growth and ageing of the world population, cancer will increase markedly in future years, in absolute numbers, and in importance relative to other diseases. We can estimate that in 2025 in the absence of any change in risk, there would be 18.5 million new cases; of the 7.7 million additional cancers, one third are due to increased number of people, but two thirds are due to population ageing.

Here is the challenge for public health – to reduce risk (so that these numbers are not attained), and to improve cancer care for the elderly in terms of survival and quality of life.

Investigating trends in cancer rates over time has important applications in epidemiological research and public health planning. Choice of data (incidence or mortality) is determined by the focus of the study, and data availability. There are various sources of bias that must be taken into account, especially when comparative studies involve different countries.

In stable populations, a change in incidence should reflect changes in exposure to environmental risk factors, and help in generating etiological hypotheses, or confirming suspected associations, when, for example, exposures to putative agents are known to be changing over time. Time trend studies are also widely used in evaluation of cancer control programmes, to study the effect of primary prevention interventions (planned or unplanned), programmes of early detection, and the efficacy of treatment protocols.

Formal quantification of the separate age-adjusted contributions of period and birth cohort may help to give insight into the underlying nature of time trends. These variables can be seen as weak proxies for events that cannot be measured directly. The examination of rates by birth cohort is an essential part of temporal analysis of diseases like cancer, for which there is a long induction phase. Changes lifestyle and environmental risk factors that affect particular generations of individuals, and presumably influence the earlier stages of carcinogenesis process, will produce cohort-specific change. Period effects may act as surrogate measures of events that quickly change incidence or mortality, such as interventions on the later stages of carcinogenesis, or artefactual influences on incidence (changes in coding practice, or in diagnostic methods); rapid alterations in mortality can be the result of an improvement in survival.

Some examples of the time trends of these major cancers are presented.

Lung cancer. Mortality data are useful for study of risk for lung cancer as a whole. International trends largely reflect past exposure to tobacco in the populations concerned. This can be seen clearly in the European countries, where variation in trends by age group and sex are predominantly cohort specific and clearly smoking-related. This makes prediction of future trends relatively straightforward. Incidence data can add a further dimension – trends by histological subtype- which are probably revealing the effects of changing composition of the cigarette.

Breast cancer. Trends in the incidence and mortality of breast cancer are the outcome of a variety of influences including screening programmes, introduced in several European countries in the late 1980s. Incidence has increased in all countries (0.8% to 2.7% annually). There are temporary increases when screening was introduced, but no evidence yet of a return to pre-screening trends. In most countries, mortality was increasing until recently, but this has ceased in most, and there is now no change (Finland, Czech Republic, Denmark, France, Italy, Norway, and Slovenia) or a significant decline (Netherlands, Slovakia, Spain, Switzerland, UK). The trends are not explicable in terms of organized screening programmes, and earlier diagnosis and improvements in therapy seem more plausible explanations.

Gastro-esophageal cancers. The near-universal decline in gastric cancer incidence and mortality is well known, and difficult to partition into cohort or period effects. Screening for the disease is almost confined to Japan, but may

have had some benefit, in detecting earlier disease and reducing mortality. On the other hand, increases have been reported from several populations in cancers localized to the gastric cardia. This may represent better specification of sub site, or classification of cancers at the gastro-esophageal junction as cardia. Incidence of adenocarcinoma of esophagus (almost all in the lower 1/3) is unequivocally increasing in several countries, probably the consequence of the obesity epidemic.
In developing countries, there is much less information on trends in incidence and mortality, although, in general, they confirm that cancers associated with "poverty" are decreasing, while those linked to "affluence" are increasing. Thus, in the world's most populous nation (China) there are declines in esophageal, stomach and cervix cancer, and increases for cancers of the lung, breast, and colon-rectum.

The impact that such changes will have on the net burden of cancer worldwide is hard to predict, although it is fairly clear that the current profile will gradually evolve to a more "western" pattern. In any case, the effects of an ageing world population will be the dominant influence on mortality trends over the next 50 years.

From causality to prevention: The case of cervical cancer

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Worldwide, cervical cancer is the second most common cancer in women, with half of million cases being diagnosed every year and over 80% of these cases occurring in developing countries. It is the second most common cause of death from cancer among young women, accounting for nearly 300,000 deaths annually. Its main public health importance lies in the fact that it affects relatively young poor women, devastating their families and being an important cause of lost years of life in the developing world. This cancer reflects more than any other cancer the substantial inequities that exist in health. Although already 166 years ago Rigoni Stern thought that a sexually transmitted agent could be linked to cervical cancer, only during the last 25 years the human papillomavirus (HPV) has been identified as main cause of this cancer and a vaccine to prevent infection has been developed.
I have the privilege of being one of the scientists that participated in this discovery. My main contribution from my former Unit of Field and Intervention Studies at the International Agency for Research on Cancer (IARC) in Lyon, France, has been to design and undertake a series of molecular epidemiologic studies in over 30 countries around the world that can be summarized as follows:

Prevalence survey of HPV types in cervical cancer
Over 1,000 women with invasive cervical cancer from 22 countries were interviewed on risk factors and underwent gynecological examination with collection of biopsies from their tumours. HPV DNA detection with PCR-based assays revealed that 99.7% of the frozen biopsies were HPV-positive. This finding led us to propose that HPV was not only the main cause of cervical cancer, but also a necessary cause (1).

Case-control studies
They included over 2,500 women with cervical cancer and a similar number of control women and were carried out in 12 countries with high, intermediate and low incidence of cervical cancer (2). Odds ratios were estimated for 30 HPV types that infect the genital tract. A very strong association (ORs > 100) was found for 15 HPV types and a weak or no association was found for 12 types and 3 types fell in an intermediate position. This lead us to propose an Epidemiological Classification of HPV types that correlated well with the phylogenetic classification based on sequencing of the L1 gene (3). These studies made an important contribution to the evaluation of the carcinogenic risk of HPV types in the IARC Monographs on HPV (Nos 64 y

90). Our case-control studies also allowed the identification of the following cofactors that acting together with HPV increase the risk of progression from HPV persistent infection to cervical cancer: tobacco, high parity, long term use of oral contraceptives and past infections with herpes simplex type 2 and Chlamydia trachomatis (4). In addition, they contributed to establish the important role of male sexual behavior in the risk of developing cervical cancer (5).

Implications.

The demonstration that infection with certain types of human papillomavirus (HPV) is not only the main cause but also a necessary cause of cervical cancer has led to great advances in the prevention of this disease on two fronts: (i) primary prevention by the use of prophylactic HPV vaccines; (ii) secondary prevention by increasing the accuracy of cervical cancer screening. Two safe and efficacious prophylactic HPV vaccines have been licensed in over 100 countries, but their high price limit their accessibility in the countries that need them most. Several studies have shown that HPV DNA detection assays are more sensitive than cytology for detection of high grade precursor lesions of the cervix (CIN2/3) and suggest that they should be used as primary screening test followed by triage with cytology or visual inspection. Evidence suggests that if the current HPV vaccines were introduced into developing countries and combined effectively with appropriate secondary cervical screening strategies, the lifetime risk of developing cervical cancer could be reduced as much as 60% (6).

1. Walboomers, J.M.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J.F., Peto, J., Meijer, C.J.L.M. & Muñoz, N. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J. Pathol., 189, 12-19.

2. Muñoz, N., Bosch, F.X., de Sanjosé, S., Tafur, L., Izarzugaza, et al. (1992) The
causal link between human papillomavirus and invasive cervical cancer: a population based case control study in Colombia and Spain. Int. J. Cancer, 52, 743 49.

3. Muñoz, N., Bosch, F.X., de Sanjosé, S., Herrero, R., et al for the IARC Multicenter Cervical Cancer Study Group (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N. Eng. J. Med, 348(6): 518-27.

4. Muñoz, N., Franceschi, S., Bosetti, C., Moreno, V., Herrero,et al., for the IARC Multicentric Cervical Cancer Study Group (2002) Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet 2002, 359, 1093-101.

5. Muñoz, N., Castellsagué, X., Bosch, F.X., Tafur, L., de Sanjosé, S., Aristizabal, N., Ghaffari, A.M. & Shah, K.V. (1996) Difficulty in elucidating the male role in Colombia, a high-risk area for cervical cancer. J. Natl Cancer Inst., 88, 1068-75.

6. Muñoz, N., Franco, E.L., Herrero, R., Andrus, J.K., de Quadros, C.A., Bosch, F.X. (2008) Recommendations for Cervical Cancer Prevention in Latin America and the Caribbean. Vaccine. 2008 Aug 19;26 Suppl 11:L96-L107.

MicroRNAs and inflammatory cytokines as biomarkers of cancer diagnosis, prognosis, and therapeutic outcome

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Free radicals are ubiquitous in our body and are generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganisms. Because free radicals can also inflict cellular damage, several defenses have evolved both to protect our cells from radicals–such as the p53 pathway and antioxidant scavengers and enzymes–and to repair DNA damage. Free radicals can cause an adaptive increase in certain of the protective base excision repair enzymes. Understanding the relationship between chronic inflammation and cancer provides insights into the molecular mechanisms involved. In particular, we highlight the interaction between nitric oxide and p53 as a crucial pathway, and the role of microRNAs and cytokines in inflammatory-mediated carcinogenesis. For example, the microRNA and inflammation-related gene expression in colon, lung and esophageal cancer can predict cancer diagnosis and patient's survival.

1. Volinia, S., Calin, G. A., Liu, C. G., Ambs, S., Cimmino, A., Petrocca, F., Visone, R., Iorio, M., Roldo, C., Ferracin, M., Prueitt, R. L., Yanaihara, N., Lanza, G., Scarpa, A., Vecchione, A., Negrini, M., Harris, C. C. and Croce, C. M.: A microRNA expression signature of human solid tumors defines cancer gene targets. Proc. Natl. Acad. Sci. USA, 103: 2257-2261, 2006.

2. Yanaihara, N., Caplen, N., Bowman, E., Kumamoto, K., Yi, M., Stephens, R. M., Okamoto, A., Yokota, J., Tanaka, T., Calin, G. A., Liu, C.-G., Croce, C. M. and Harris, C. C.: microRNA expression signature predicts lung cancer diagnosis and prognosis. Cancer Cell 9: 189-198, 2006.

3. Seike, M., Yanaihara, N., Bowman, E. D., Zanetti, K. A., Budhu, A., Kumamoto, K., Mechanic, L. E., Matsumoto, S., Yokota, J., Shibata, T., Sugimura, H., Gemma, A., Kudoh, S., Wang, X. W. and Harris, C. C.: Use of a cytokine gene expression signature in lung adenocarcinoma and the surrounding tissue as a prognostic classifier. J. N. C. I., 99: 1257-1269, 2007.

4. Hussain, S. P. and Harris, C. C.: Inflammation and cancer: An ancient link with novel potentials. Int. J. Cancer, 121: 2373-2380, 2007.

5. Schetter, A. J., Leung, S. Y., Sohn, J. J., Zanetti, K. A., Bowman, E. D., Yanaihara, N., Yuen, S. T., Chan, T. L., Kwong, D.L.W., Au, G. K. H., Liu, C. G., Calin, G. A., Croce, C. M. and Harris, C. C.: MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA, 299: 425-436, 2008.

Nutritional energy balance and cancer risk: Epidemiological evidence implicating insulin and insulin-like growth factor-I

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Epidemiological observations strongly implicate nutritional energy balance as a key risk factor for cancer development. Anthropometric indices of excess body weight are associated with increased risks of cancers of the endometrium, breast (postmenopausal women), kidney (renal cell tumours), colon, pancreas and oesophagus (adenocarcinomas). By contrast, regular physical activity reduces the risk of developing breast and colorectal cancers, and potentially other tumour types. Overall, excess weight and lack of physical activity may account for one quarter to half of the occurrence of the above-mentioned tumour types.

One physiological major mechanism that may provide a metabolic link between excess body weight, physical inactivity and increased cancer risks is the increase in blood and tissue levels of insulin and insulin-like growth factor-I. Besides their classical metabolic effects, insulin and IGF-I both have major growth factor activities, stimulating cell growth and proliferation, and inhibiting apoptosis in a great variety of tissue and cell types.

Excess body weight and low physical activity leads to a reduced sensitivity, which in turn is compensated for by increased fasting and postprandial insulin levels in the circulation. Endogenous insulin levels are also a key regulator of liver synthesis and blood levels of insulin-like growth factor-I, and of IGF-binding proteins -1 and -2. In energy restricted states, as well in type-1 diabetes, endogenous insulin levels are low and the growth hormone stimulated synthesis of IGF-I in the liver is inhibited, leading to reduced circulating IGF-I levels. Furthermore, at low pancreatic insulin secretion there is an increased production of IGFBP-1 and IGFBP-2, further diminishing the availability of IGF-I to tissue receptors. By contrast, in obese and hyperinsulinemic states, free circulating IGF-I levels are increased, even though total IGF-I levels may be reduced compared those in well-nourished but normal-weight subjects.

Prospective cohort studies have shown increased risks particularly of colon cancer and endometrial cancer among women and men with high fasting and non-fasting plasma insulin concentrations, and similar associations have been reported for pancreas cancer. Likewise, pooled analyses of prospective cohort study data show a (moderately) increased risk of cancers of the prostate and breast, in particular, among individuals that have comparatively elevated plasma concentrations of IGF-I.

In addition to insulin, large-scale prospective studies have shown increased risks of cancers of the pancreas, liver and endometrium, as well as of the colon, among women and men who had comparatively elevated fasting plasma glucose concentrations. Among patients with type-2 diabetes, recent epidemiological studies showed a reduced overall cancer occurrence in association with the use of metformin as the major treatment, compared to treatments that raise insulin levels. Metformin has strong glucose and insulin-lowering effects. In addition, however, there is evidence from recent experimental studies that tumour inhibiting effects of metformin could also be mediated directly at the level of (tumour) target tissues, by suppression of AMP-activated kinase (AMPK) activity. AMPK plays a central role in the regulation of anabolic versus catabolic processes as a function of cellular energy status.

Apart from insulin, glucose and IGF-I, there is strong evidence that effects of nutritional energy balance on tumour development can be mediated by alterations in available endogenous sex hormones (e.g. cancers of the endometrium and breast), or by the induction of a chronic state of low-grade inflammation (e.g. colon cancer).

Gaining a better understanding of the mechanisms relating excess weight and physical inactivity to cancer may lead to improved strategies for both cancer prevention and treatment.

Calle EE, Kaaks R.
Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms.Nat Rev Cancer. 2004;4:579-91.

Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD.
Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;330:1304-5.

Kaaks R, Lukanova A.
Energy balance and cancer: the role of insulin and insulin-like growth factor-I.
Proc Nutr Soc. 2001;60:91-106.

Jenab M, Riboli E, Cleveland RJ et al.
Serum C-peptide, IGFBP-1 and IGFBP-2 and risk of colon and rectal cancers in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2007;121:368-76

Roddam AW, Allen NE, Appleby P et al.
Insulin-like growth factors, their binding proteins, and prostate cancer risk: analysis of individual patient data from 12 prospective studies. Ann Intern Med. 2008;149:461-71.

Changing cancer mortality

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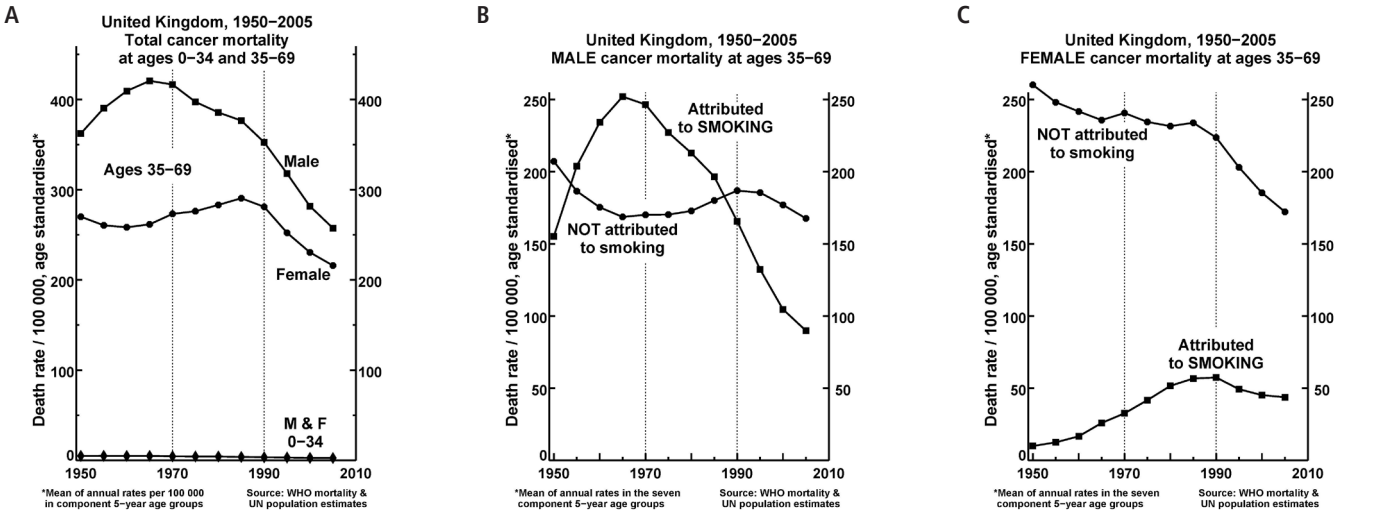
Around the middle of the 20th century it was found that tobacco was a cause of most of the lung cancer deaths in Europe and North America, and that it also caused smaller numbers of deaths from other types of cancer (1). Since then, fluctuations in the national cancer mortality trends in those continents, particularly among men, have been dominated by large increases and then, in some countries, large decreases in tobacco-attributed cancer mortality.2 (This includes most, though not all, of the lung cancer deaths, plus a smaller number of other cancer deaths.)

In 1970 the worst-affected country in the world was the UK, where tobacco was responsible for about 60% of all male cancer deaths at 35-69 years of age, plus an increasing number of female cancer deaths. Since 1970 there has been a substantial decrease in UK male cancer mortality at these ages, due mainly to the substantial decrease in tobacco-attributed cancer mortality at these ages (2) (see graphs: death from cancer before age 35 is uncommon). UK male cancer death rates from tobacco are now less than half of what they were in the 1960s, and UK female cancer death rates from tobacco are now less than half of what they would have been if women had continued. smoking as they were in the 1960s. Still, however, smoking remains the single most important cause of cancer in such countries (3).

Over the past half-century the cancer death rate among UK non-smokers has changed relatively little (as indicated in the graphs by the mortality from cancer that is not attributed to smoking). Nevertheless, there have been moderate decreases in non-smoker cancer mortality since 1990, partly due to the delayed effects of poorly understood decreases in the causes of cancer of the stomach, intestine and uterus, partly due to more screening for precancerous lesions of the uterus, and partly due to earlier detection and better treatment (particularly of breast cancer (4), where the effects of several moderate improvements have in recent years combined to reduce UK breast cancer mortality by more than one-third).

Reductions in the adverse effects of smoking in some developed countries are, however, outweighed by the growing effects of cigarette and bidi consumption in large populations elsewhere, such as those of China and India. Tobacco caused about 100 million deaths in the 20th century and, if current smoking patterns persist (with widespread uptake and little cessation), it will cause about 1000 million deaths in the present century (5,6).

Legend to figure: United Kingdom, 1920-2005. (a) Total annual cancer mortality rates as ages 0-34 and 35-69 years, with (b) the total male and (c) the total female rates at ages 35-69 subdivided into the parts attributed, and not attributed, to smoking.2 Note: An annual rate of 300 per 100,000 corresponds to about a 10% risk of death over a 35-year age range.



1. Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ 2004; 328: 1519-27

2. Peto R, Lopez A, Boreham J, Thun M. Mortality from smoking in developed countries, 1950-2000 (2nd edition), available on www.deathsfromsmoking.net. [Contains many graphs and tables for the UK, USA, EU and 40 countries.]

3. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 1981; 66: 1191-1308

4. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005; 365: 1687-1717

5. The hazards of smoking and the benefits of stopping: cancer mortality and overall mortality. Pages 15-27 in: International Agency for research on Cancer. Reversal of risk after quitting smoking. Lyon, IARC, 2007

6. WHO report on the global tobacco epidemic, 2008: the MPOWER package. Geneva, World Health Organisation, 2008

Diagnostic and therapeutic discoveries from genome-scale functional and translational cancer research

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We are applying an integrated systems approach for the systematic rapid exploration of the function and clinical significance of all genes and signalling pathways in cancer. The development of novel technologies is essential for translational cancer genomics.

First, we have developed an ultra-high density cell-microarray screening system for siRNAs and miRNAs. The cell array technology has up to 100-fold screening throughput as compared to 384-well-based assays. In this technology, siRNAs and transfection agents are first printed as a microarray with up to 10-20,000 spots per array. Cultured cells are then allowed to adhere on top of the array, where they undergo transfection with the siRNAs in a spatially confined manner. Cell phenotypes resulting from the knockdown of specific genes are read with HTS and HCS instrumentation using up to 4 parameters at a time. Our primary applications include identification of genes whose knockdown causes specific cancer cell phenotypes, with or without drug treatment (synthetic lethal screening), such as induction of apoptosis and activation of oncogenic signalling. Examples from screening of epigenetic endpoints and miRNAs regulating ER protein will be shown.

Second, we have developed an in silico transcriptomics data mining capability for the rapid analysis of gene expression levels in vivo in thousands of clinical samples, which is available for research purposes at www.genesapiens.org (Kilpinen et al.. Genome Biology, 2008). The transcriptomics data originate from a collection of publicly available gene expression data covering over 14,000 samples from >150 normal and disease tissues. The data have been normalized, QC-checked and annotated with clinical information to generate a fully integrated, curated and searchable database to systematically explore gene functions across the body in different cells, tissues and diseases, including cancer. GeneSapiens data mining makes it possible to carry out rapid comprehensive analyses and validation of the clinical role of individual genes, therapeutic and diagnostic targets, gene sets or pathways in cancer, across all tissues and tumor types.

Taken together, the cell array and GeneSapiens transcriptomics approaches provide powerful new tools for genome-scale translational cancer research.

The Cancer Genome Atlas (TCGA) Project

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The Cancer Genome Atlas (TCGA) is a comprehensive team science project to accelerate our understanding of human cancer through a coordinated, large-scale effort to systematically explore the entire spectrum of cancer-associated genomic changes. TCGA is jointly sponsored by The National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) as a public service project, in the manner of the Human Genome Project. The project is organized around the principle of simultaneous, coordinated application of cutting-edge genome analysis technologies, including high-throughput DNA sequencing, to the same tumor samples so that all data types can be directly compared. The overarching goal of TCGA is to improve cancer medicine through a greater understanding of the specific genomic/molecular characteristics of cancer types and subtypes. Detailed information about TCGA can be found at: http://cancergenome.nih.gov

Specifically, TCGA analysis includes characterization of DNA copy number changes, including large (on the order of chromosome segments) and small (1,000–100,000 kb) scale rearrangements, gene transcription profiling, epigenetic modifications, and sequence variation. The entire suite of analysis platforms is applied to the interrogation of a common set of molecular analytes obtained from clinically annotated, high-quality tumor biospecimens and matched normal control biospecimens; i.e., normal tissue or blood. The analysis data is put into the public domain in order to provide the scientific community with new tools for cancer research.

During the 3-year pilot phase of TCGA, running from October 2006 through September 2009, the project already has been shown to be an overwhelming success. Three cancers were chosen for the pilot project: glioblastoma multiforme (GBM), serous cystadenocarcinoma of the ovary, and squamous cell carcinoma of the lung. As delineated in the first TCGA publication on glioblastoma (Nature 2008; 455: 1061-8), the integrated analysis of chromosomal copy number, gene expression, and DNA methylation data provided new insights into the roles of human epidermal growth factor receptor 2 (ERBB2), neurofibromatosis 1 (NF1), and tumor protein 53 (TP53) in this disease. The data showed that alterations in three signaling pathways were identified as being central to the development of the disease: namely, the receptor tyrosine kinase (RTK), the TP53, and the retinoblastoma (RB) tumor suppressor pathways. In addition, frequent mutations were found in the regulatory subunit of the phosphoinositide-3-kinase, regulatory subunit 1 (PIK3R1) gene.

TCGA is one of the most ambitious scientific undertakings and community resource projects ever undertaken by the NCI. The study incorporates an extraordinary array of scientific expertise and analytical platforms to integrate clinical, pathological, and genomic analysis data and to make these data freely and publicly available in order to advance cancer medicine. The improved understanding of cancer biology may lead to new targets for cancer therapeutics, better tools for assigning patients to clinical trials, more personalized treatment plans for each patient, better diagnostics, a more accurate basis for assessing risk of acquiring specific cancers, and improved strategies for cancer prevention.

Cancer Genome Atlas Research Network (2008)
Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature.455 (7216): 1061-1068

The von Hippel-Lindau tumor suppressor gene: Oxygen sensing and cancer

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Inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene plays an important role in clear cell renal carcinoma, hemangioblastoma, and pheochromocytoma (intraadrenal paragangliomas). Individuals with germline VHL mutations (VHL disease) are at increased risk for these tumors in an allele-specific manner (genotype-phenotype correlation). The VHL gene product (pVHL) has multiple functions including acting as the substrate recognition subunit of an E3 ubiquitin ligase that targets the alpha subunits of the heterodimeric transcription factor HIF (Hypoxia-inducible Factor) for destruction. HIFa must be hydroxylated on one (or both) of two conserved prolyl residues by members of the EglN family (also called PHD or HPH family), which are oxygen-dependent enzymes that also require reduced iron, 2-oxoglutarate, and ascorbic acid, in order to bind to pVHL. Under low oxygen conditions, or in cells lacking wild-type pVHL, HIFa accumulates and activates 100-200 genes involved in adaptation to hypoxia. Deregulation of HIFa (especially HIF2a) appears to play a causal role in clear cell renal carcinoma and almost certainly contributes to the development of hemangioblastomas, which are blood vessel tumors. In contrast, deregulation of HIFa does not appear drive the development of pheochromocytoma. In particular, some VHL families have VHL alleles that are essentially wild-type with respect to HIFa regulation and present with familial pheochromocytoma.

Higher metazoans, including people, have three EglN family members (EglN1, EglN2, and EglN3). We made a conditional EglN1 mouse (EglN1-/- embryos are not viable) and confirmed cell culture experiments that suggested EglN1 (PHD2) is the primary HIF prolyl hydroxylase. Our recent studies suggest that EglN2 and EglN3 play roles in control of cell proliferation and apoptosis, respectively. We found, for example, that the genes that, when mutated, cause familial paraganglioma define a pathway that is activated in sympathetic neuroblasts during embryological development by growth factor withdrawal. Interestingly, this pathway impinges upon EglN3, which is both necessary and sufficient for apoptosis in this setting. EglN3 can also induce apoptosis in a variety of tumor cell types of both neuronal and non-neuronal origin. In an unbiased screen for shRNAs that confer protection against EglN3-induced apoptosis, we identified an shRNA directed against KIF1Bb, which maps to 1p36.2. This region of the genome is frequently deleted in a variety of tumors, including neuroblastoma.

Notably, this gene is also one of only 6 annotated genes located within a 500 kB homozygous deletion in a neuroblastoma line. Restoration of KIF1Bb function in this line induces apoptosis and we have identified germline loss of function KIF1Bb mutations in some neuroblastoma and pheochromocytoma patients, arguing that KIF1Bb is a potential tumor suppressor gene. Preliminary data suggest that KIF1Bb haploinsuffiency is sufficient to protect from apoptosis, which might account for the observation that many 1p deleted tumors retain a wild-type KIF1Bb allele.

1. Minamishima, Y.A., et al., Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure. Blood, 2008. 111: p. 3236-3244.

2. Schlisio, S., et al., The kinesin KIF1Bbeta acts downstream from EglN3 to induce apoptosis and is a potential 1p36 tumor suppressor. Genes Dev, 2008. 22(7): p. 884-93.

3. Kaelin, W.G., Jr. and P.J. Ratcliffe, Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell, 2008. 30(4): p. 393-402.

4. Kaelin, W.G., Jr., The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. Nat Rev Cancer, 2008. 8(11): p. 865-73.

How metastatic cells usurp innate immunity and inflammatory processes

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There is ample epidemiological and mechanistic evidence that inflammation and inflammatory processes, such as these that lead to activation of NF-κB and STAT3, play critical role in early tumor promotion and the growth and progression of primary tumors. Little information, however, exists regarding the role of inflammation in metastatic progression, which until recently was mainly attributed to genetic changes intrinsic to the cancer cell. Using a mouse model of prostate cancer metastatic progression, the TRAMP mouse, we found that activation and nuclear translocation of IKB kinase a (IKK a) within prostate cancer (CaP) cells is a critical event in metastatogenesis, as it is required for repression of the potent metastasis suppressor maspin. Activation of IKKa in CaP cells, however, depends on interaction with inflammatory cells that are recruited into the growing tumors and produce IKKa activating cytokines such as RANK ligand and lymphotoxin. Interestingly, expression of IKKa activating cytokines is dependent on IKKb. Furthermore, androgen ablation therapy (such as castration) results in activation of IKKb in immune cells that are recruited to the dying tumor and IKKa in the residual CaP cells that survive, leading to emergence of hormone-independent CaP.

To understand how inflammatory cells are recruited into growing tumors to promote metastatic progression, we screened carcinoma lines for their ability to produce soluble factors that activate macrophages and induce cytokine production. We identified one such factor as the extracellular matrix protein versican, whose expression is highly elevated in aggressive lung cancers, and have shown that it activates macrophages through TLR2 to produce TNF-a and other inflammatory cytokines. Most importantly, the ability of lung carcinoma cells that produce versican to establish metastatic growths is strongly dependent on TLR2 activation and TNF-a production by host bone-marrow derived cells.

These results strongly support the notion that metastatic progression is highly dependent on dynamic and reciprocal interactions between cancer cells and inflammatory cells, which are recruited into growing tumors to produce pro-metastatic cytokines.

1. Karin, M., Cao Y., Greten, F., and Li, Z-W. (2002) NF-kB in Cancer: From innocent bystander to major culprit. Nature Rev Cancer 2:301-10.

2. Cao, Y., Bonizzi, G., Seagroves, T., Greten, F., Johnson, R., Schmidt, E., Karin, M. (2001) IKKa provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. Cell 107:763-775.

3. Luo, J.-L., Tan, W., Ricono, J.M., Korchynskyi, O., Zhang, M., Gonias, S.L., Cheresh, D.A., Karin, M. (2007). Nuclear cytokine activated IKKa controls prostate cancer metastasis by repressing maspin. Nature 446: 690-4.

4. Kim, S., Takahashi, H., Lin, W., Descargues, P., Grivennikov, S., Kim, Y., Luo, J., Karin, M. (2008) Carcinoma produced factors activate myeloid cells via TLR2 to stimulate metastasis. Nature. 2009 Jan 1;457(7225):102-6.

Apoptosis proteins as targets for anticancer therapy

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Evasion of apoptosis (programmed cell death) is a hallmark of human cancers (1). Further, most anticancer therapies that are commonly used in the clinic, e.g. chemo- or radiotherapy, primarily act by inducing cell death pathways including apoptosis in cancer cells (2). Thus, failure to undergo apoptosis may result in primary or acquired resistance of cancers to current treatment approaches. Understanding the molecular events that regulate apoptosis in response to anticancer therapy and how cancer cells evade apoptotic cell death provides novel opportunities for the development of molecular therapeutics that target cell death pathways. Studies over the last decade have delineated multiple defects in the apoptosis signal transduction machinery that can serve as targets for the design of novel treatment strategies (3). The enormous progress in apoptosis research has recently started to be translated into clinical application. Examples for apoptosis targeted cancer therapeutics that have already entered the clinical stage include TRAIL receptor agonists, which directly trigger programmed cell death, or small molecule inhibitors of antiapoptotic proteins such as "Inhibitor of Apoptosis Proteins" (IAPs) or Bcl-2 family proteins. Further insights into the mechanisms of tumor resistance to apoptosis are expected to turn into benefit for patients suffering from cancer.

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell, 100, 57-70, 2000.

2. Fulda S, Debatin KM. Therapeutic Modulation of Apoptosis in Cancer Therapy. In: Fulda S, Debatin KM, editors. Apoptosis and Cancer Therapy. Weinheim: Wiley-VCH; 2006. p. 515-535.

3. Fulda S. Tumor resistance to apoptosis. Int J Cancer. 2009 Feb 1;124(3):511-5.

Repair of oxydative damage

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Background
The maintenance of genetic stability is of crucial importance for any form of life. Genomic instability can lead to death and/or cancer. On the other hand DNA itself is highly reactive and is constantly attacked by reactive oxygen species from in and outside the cell. 7,8-dihydro-8-oxoguanine (8-oxo-G) is recognized as the most important oxidative DNA lesions because of its mutagenic potential. The steady-state level of 8-oxo-G lesions is about 103 per normal cell and up to 105 lesions in cancer cells. All DNA polymerases (pols) studied so far show significant error-prone bypass of 8-oxo-G, since they preferentially incorporate a wrong Adenine opposite an 8-oxo-G instead of the correct Cytosine.

Findings and Relevance
Specialized pols are required for translesion synthesis. Auxiliary factors play an important, but so far poorly understood, role in this process. The effects of proliferating cell nuclear antigen (PCNA) and replication protein A (RP-A) on six different human pols belonging to the B, Y and X families, were analyzed during in vitro bypass of different lesions. A major and specific effect was found for pol l (1). PCNA and RP-A allowed the correct incorporation of dCTP by pol l opposite a template 8-oxo-G 1200- fold more efficiently than the incorrect dATP. These findings suggested a novel accurate mechanism to reduce the deleterious consequences of oxidative damage and, in addition, point to an important role for PCNA and RP-A in determining a functional hierarchy among different pols in 8-oxo-G bypass. The adenine misincorporated by replicative pols opposite 8-oxo-G is removed by a specific glycosylase, leaving the lesion on the DNA. Subsequent incorporation of C opposite 8-oxo-G on the resulting one nucleotide gapped DNA is essential for the removal of the 8-oxo-G to prevent G-C to T-A transversion mutations. By using model DNA templates, purified pols b and l and knockout cell extracts, we show that the auxiliary proteins RP-A and PCNA act as molecular switches to activate the pol l- dependent highly efficient and faithful repair of A:8-oxo-G mismatches in human cells and to repress pol b activity. By using an immortalized human fibroblast cell line that has the potential to induce cancer in mice, we show that that the development of a tumor phenotype in these cells correlated with a differential expression of pols l and b (2, 3).

Moreover, we identified pol l as an interaction partner of cyclin-dependent kinase 2 (Cdk2) that is central for the cell cycle G1/S transition and S phase progression. This interaction leads in vitro to phosphorylation of pol l, and its in vivo phosphorylation pattern during cell cycle progression mimics the modulation by Cdk2/cyclin A (4). Four different phosphorylation sites were identified for pol l. Experiments with phosphorylation-defective mutants suggested that phosphorylation of T553 is critical for maintaining pol l stability, since it is targeted to the proteasomal degradation pathway via ubiquitination unless this residue can be phosphorylated. In particular, pol l is stabilized during cell cycle progression in late S and G2 phase. This likely enables pol l to properly conduct repair of 8-oxo-G damaged DNA these phases before cells enter mitosis thus preventing G-C->T-A transversion mutations (5).

1. Maga, G., Villani, G., Crespan, E., Wimmer, U., Ferrari, E. Bertocci, B. and Hübscher, U.: Nature, 447, 606-609, 2007.

2. Maga, G., Crespan, E., Wimmer, U., van Loon, B., Amoroso, A., Mondello, C., Ferrari, E., Belgiovine, C.,,Villani, G., Hübscher, U.: Proc Natl Acad Sci U S A. 2008 Dec 30;105(52):20689-94. Epub 2008 Dec 22.

3. Amoroso, A., Wimmer, U., Hübscher, U. and Maga, G.: Curr. Mol. Pharmacol., 1, 162-170, 2008

4. Frouin, I., Toueille, M., Ferrari, E., Shevelev, I. & Hübscher, U.: Nucl. Acids Res. 33, 5354-5361, 2005.

5. Wimmer, U., Ferrari, E., Hunziker, P. and Hübscher, U.: EMBO reports, 9, 1027-1033, 2008

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Micro-RNA mediated suppression of tumorigenesis

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Progress in understanding the biology of multiple myeloma (MM), a plasma cell malignancy, has been slow. The discovery of microRNAs (miRNAs), a class of small noncoding RNAs targeting multiple mRNAs, has revealed a new level of gene expression regulation. To determine whether miRNAs play a role in the malignant transformation of plasma cells (PCs), we have used both miRNA microarrays and quantitative real time PCR to profile miRNA expression in MM-derived cell lines (n = 49) and CD138+ bone marrow PCs from subjects with MM (n =16), monoclonal gammopathy of undetermined significance (MGUS) (n = 6), and normal donors (n = 6). We identified overexpression of miR-21, miR-106b~25 cluster, miR-181a and b in MM and MGUS samples with respect to healthy PCs. Selective up-regulation of miR-32 and miR-17~92 cluster was identified in MM subjects and cell lines but not in MGUS subjects or healthy PCs. Furthermore, two miRNAs, miR-19a and 19b, that are part of the miR-17~92 cluster, were shown to down regulate expression of SOCS-1, a gene frequently silenced in MM that plays a critical role as inhibitor of IL-6 growth signaling. We also identified p300-CBP-associated factor, a gene involved in p53 regulation, as a bona fide target of the miR106b~25 cluster, miR-181a and b, and miR-32. Xenograft studies using human MM cell lines treated with miR-19a and b, and miR-181a and b antagonists resulted in significant suppression of tumor growth in nude mice. In summary, we have described a MM miRNA signature, which includes miRNAs that modulate the expression of proteins critical to myeloma pathogenesis.

Calin GA, et al. (2005) A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 353:1793–1801.

Garzon R, et al. (2008) MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood 111:3183–3189.

Croce CM (2008) Oncogenes and cancer. N Engl J Med 358:502–511.

Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6:857–866.

Cancer stem cells in melanoma: Potential culprits for tumor growth and metastasis

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Evidence is accumulating that cancer stem cells, though rare within a tumor, have the capacity to initiate and sustain tumor formation and expansion (1). Their specific elimination might therefore represent an efficient tumor therapy. To achieve this goal it is necessary to determine the characteristics of cancer stem cells, which likely depend on the tumor's origin. Normally, tissue-specific stem cells are implicated in the generation, homeostasis and regeneration of particular organs. In analogy, the cancer stem cell concept predicts that particular cancers arise from specific cancer stem cell types. In our studies we aimed to apply this concept to melanoma, the most fatal skin tumor. During embryonic development, neural crest stem cells give rise to pigmented melanocytes in the skin (2). Melanocytic cells, on the other hand, can form melanoma upon oncogenic mutations (3). Therefore we predicted that putative melanoma stem cells might share properties of neural crest-derived stem cells present in the adult skin (4, 5). Indeed, we were recently able to identify melanoma stem cells that express markers of neural crest stem cells and, similar to their normal counterparts, are able to self-renew and to generate multiple cell types. Intriguingly, the frequency of these cells in human melanoma correlates with metastasis and poor prognosis and might thus serve as a novel prognostic tool. Transplantation of melanoma stem cells, even at low cell numbers, invariably leads to the formation of heterogeneous tumors resembling the parental tumor and containing stem cells again. Finally, forced differentiation decreasing stem cell activity leads to reduced tumor growth. Our findings demonstrate that targeting melanoma stem cells might overcome the present stand still in melanoma therapy. Thus, having identified melanoma stem cells, efforts can and should be made to develop drugs able to promote their differentiation, to specifically block their self-renewal, or to selectively kill these cells.

1. Ward, R., and Dirks, P. 2007. Cancer Stem Cells: At the Headwaters of Tumor Development. Annu. Rev. Pathol. 2:175-189.

2. Sommer, L. 2005. Checkpoints of melanocyte stem cell development. Science's STKE, 2005 (298), pp. pe42.

3. Zabierowski, S.E., and Herlyn, M. 2008. Melanoma stem cells: the dark seed of melanoma. J. Clin. Oncol. 26:2890-2894.

4. Nishimura, E.K., et al. 2002. Dominant role of the niche in melanocyte stem-cell fate determination. Nature 416:854-860.

5. Wong, C.E., et al. 2006. Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin. J. Cell. Biol. 175:1005-1015.

Cutaneous cancer stem cells

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Continuous turnover of epithelia is ensured by the extensive self-renewal capacity of tissue specific stem cells1. Likewise, many carcinomas contain a subpopulation of cells which co-opt stem cell properties2,3. Whether tissue specific stem cells are also at the origin of tumorigenesis and directly give rise to cancer stem cells (CSC) is an open question for many cancers. In murine skin, follicular morphogenesis is driven by stem cells located in the bulge of the follicle. We now provide evidence that these stem cells are the target cells for mutation giving rise to squamous cell carcinomas and that these bulge stem cells are the direct ancestors of cancer stem cells of this tumor4. These cancer stem cells maintain phenotypic and functional similarities to normal bulge skin stem cells and are the only cells with tumor initiation properties. Transplants derived from these CSCs preserve the hierarchical organization of the primary tumor. Importantly, we describe beta-catenin signaling as essential in sustaining the cancer stem cell phenotype. Ablation of the beta-catenin gene results in the loss of CSCs followed by complete tumor regression. Similarly, we provide evidence for an important role of increased beta-catenin signaling in malignant human squamous cell carcinomas (SCC). As Wnt/beta-catenin signaling is not essential for normal epidermal homeostasis5, such a mechanistic difference may thus be targeted to eliminate cancer stem cells and consequently eradicate SCC.

1. Blanpain, C., V. Horsley, E. Fuchs (2007). Epithelial stem cells: turning over new leaves. Cell. 128(3), 445-58.

2. Malanchi, I., J. Huelsken (2009). Cancer stem cells: never Wnt away from the niche. Curr. Opin. Oncol. in print.

3. Reya, T., S.J. Morrison, M.F. Clarke, I.L. Weissman (2001). Stem cells, cancer, and cancer stem cells. Nature 414, 105-11.

4. Malanchi, I., H. Peinado, D. Kassen, D. Metzger, P. Chambon, M. Huber, D. Hohl, A. Cano, W. Birchmeier, J. Huelsken (2008). Cutaneous cancer stem cell maintenance is dependent on b-catenin signaling. Nature 452, 650-53.

5. Huelsken, J., R. Vogel, B. Erdmann, G. Cotsarelis, W. Birchmeier (2001). b-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell 105, 533-45.

Molecular analysis of normal and malignant epithelial stem cells

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Most common cancers, such as cancers of the breast and colon, arise in organs such as the breast that contain a small population of stem cells that constantly replenish the mature cells of the tissue. Stem cells are defined by the ability to divide and give rise to a new stem cell (self-renewal), as well as the ability to give rise to the differentiated cells of an organ, and thus are the only long-lived cell population in many tissues. Epithelial tumors consist of a heterogeneous population of cancer cells that differ in their apparent state of differentiation, suggesting that solid tumors might represent aberrant organs containing a cancer stem cell population that maintains the ability to self-renew. Indeed, using a xenograft model of human breast, colon and head and neck cancers, a phenotypically-distinct subset of the cancer cells (cancer stem cells) has been found to have the exclusive ability to form tumors. The remaining cancer cells, which often form the bulk of the tumor, are unable to self-renew or sustain tumorigenesis. Recently, it has become apparent that some oncogenes and tumor suppressor genes also regulate self-renewal, the process by which both normal and malignant stem cells maintain themselves. The process of self-renewal is de-regulated in cancer stem cells resulting in tumor formation. Extensive molecular and cellular analyses of normal and malignant breast, head and neck, and colon stem cells will be discussed. These analyses have revealed new pathways that regulate self renewal as well as resistance to cytotoxic therapies in normal and cancer stem cells.

Poster Abstracts

1

Tumor recovery by angiogenic switch from sprouting to intussusceptive angiogenesis

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Background. Radiation and inhibitors of angiogenesis induce compensatory changes in the tumor vasculature not only during, but also after the treatment cessation.
Methodology. Mammary carcinoma allografts were investigated by vascular casting, electron, light, confocal microscopy and immunoblotting after fractionated irradiation or treatment with the VEGF-receptor tyrosine kinase inhibitor, PTK787/ZK222854.
Results. Irradiation and anti-angiogenic therapy had similar effects on the tumor vasculature in the recovery phase. Both treatments reduced microvascular density, particularly in the tumor medulla. After cessation of therapy, the tumor vasculature expanded predominantly by intussusception with a plexus composed of enlarged sinusoidal-like vessels containing multiple transluminal tissue pillars. Tumor revascularization originated from preserved SMA-positive vessels in tumor cortex. Quantification revealed that recovery was characterized by an angiogenic switch from sprouting to intussusception. The upregulated SMA-expression during the recovery reflected the recruitment of SMA-positive cells for intussusception as a part of angioadaptive mechanism. Tumor recovery was associated with a dramatic decrease (by 30-40%) in the intratumoral microvascular density, probably as result of intussusceptive pruning, surprisingly with only a minimal reduction of the total microvascular area . Therefore, the vascular supply to the tumor was sufficient as corroborated by HIFα immunostaining.
Conclusion. Irradiation and anti-angiogenic therapy induce a switch from sprouting to intussusceptive angiogenesis as part of a compensatory response to preserve and restore perfusion. Intussusceptive angiogenesis with an associated low endothelial proliferation rate and permeability, may represent an escape mechanism and account for the development of resistance to therapy, as well as the rapid recovery of tumor vasculature after cessation of therapy.

2

Functional polymorphic variants of methionine metabolism influence outcome in a population of patients with primary central nervous system lymphoma (PCNSL) treated with high-dose methotrexate

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This study aimed at analyzing the impact of eight genetic variants of methionine metabolism on efficacy and side-effects of methotrexate. Of 65 patients with primary central nervous system lymphoma (PCNSL), treated with methotrexate-based polychemotherapy, 20 developed confluent CNS white matter changes (WMC). The occurrence of WMC was significantly predicted by the genotype of methylenetetrahydrofolate reductase c.1298A>C (p.E429A; patients with WMC, AA/AC/CC: 0.80/0.10/0.10; patients without WMC: 0.40/0.47/0.13; nominal logistic regression: Chi-square=11.54; p=0.003). In addition, the transcobalamin 2 variant c.776C>G (p.P259R) influenced both the occurrence of WMC (patients with WMC: 0.20/0.45/0.35; patients without WMC: 0.33/0.47/0.20; Chi-square=11.15; p=0.003) as well as the mean overall survival (c.776CC: 105plusminus7 months; c.776CG/GG: 69plusminus9 months; Log Rank 4.79; p=0.029; Wald 4.15; p=0.042; median overall survival not yet reached).
These data suggest that functional variants of methionine metabolism influence the efficacy and neurotoxicity of methotrexate. The mutant alleles of MTHFR c.677C>T and Tc2 c.776C>G are known to be associated with reduced synthesis of methionine and S-adenosylmethionine, which is required for CNS myelination. This may increase the depletion of S-adenosylmethionine caused by methotrexate explaining the association of these variants with WMC during methotrexate-therapy. Further, these variants influence the metabolism of tetrahydrofolates required for nucleic acid synthesis. This may modify the pharmacological effect of methotrexate on nucleic acid and may explain the association of the Tc2 variant with overall survival. As methionine metabolism can be manipulated easily, e.g. by supplementation of vitamins or of S-adenosylmethionine, these data may lead to novel strategies to improve methotrexate efficacy and side effects.

3

The DEAH helicase RHAU is essential for the growth of ras-transformed MEF cells

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The DEAH-box RNA helicase RHAU was identified in our lab as one of regulatory molecules of uPA mRNA stability. RHAU is mainly localized in the nucleus and regulation of those genes whose expression is modified by its knockdown in HeLa cells is mostly transcriptional. There is no RHAU orthologs either in yeast or C. elegans and no report on the function of RHAU ortholog in Drosophila, making it difficult to speculate its biological function. Therefore, in order to elucidate its biological role we established an immortalized mouse embryonic fibroblast cell line in which the RHAU gene is floxed by two loxP sites and tamoxifen-inducible Cre is constitutively expressed. After RHAU knockout these cells showed a reduced proliferation rate, however, they still did grow without obvious morphology changes. Microarray analysis of global gene expression in these cells revealed that many genes are regulated by RHAU, among which most prominent are those involved in cell growth/oncogenesis . Analysis of miRNAs showed 10 different miRNAs (5 up and 5 down) after RHAU knockout, among which some are involved in oncogenesis. These results suggest functional interaction between transformation and RHAU. To investigation this possibility, the MEF cells were infected by a ras-expressing virus-based vector. When RHAU knockout was induced in ras-transformed MEF cells, cells stopped proliferation completely, while non-transformed cells still continued to proliferate, indicating that ras-transformation renders RHAU essential for MEF cell proliferation.

1. Iwamoto F, Stadler M, Chalupnikova¡ K, Oakeley E, Nagamine Y (2008) Transcription-dependent nucleolar cap localization and possible nuclear function of DExH RNA helicase RHAU. Exp Cell Res.: 314(6):1378-1391
2. Creacy SD, Routh ED, Iwamoto F, Nagamine Y, Akman SA, Vaughn JP. (2008) G4 resolvase-1 binds both DNA and RNA tetramolecular quadruplex with high affinity and is the major source of tetramolecular quadruplex G4-DNA and G4-RNA resolving activity in HeLa cell lysates. J Biol Chem. 2008 Dec 12;283(50):34626-34. Epub 2008 Oct 7.

4

Investigation of the molecular determinants of rapamycin sensitivity in acute myeloid leukemia cells

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In acute myeloid leukemia (AML) cells, protein expression analysis revealed significant differences in the expression of the mammalian target of rapamycin (mTOR). Therefore, the role of mTOR on AML cell growth and survival was investigated and cellular responses to the mTOR inhibitor rapamycin were compared in mTORhigh and mTORlow cells. Moreover, an RNAi screen was aimed at uncovering kinases which modulate the sensitivity to rapamycin.

AML cell lines and blasts were screened for mTOR protein expression. Rapamycin and small interfering RNA (siRNA) were used to inhibit mTOR. A siRNA library targeting various human kinases was used for an RNAi screen.

The mTOR protein was found to be expressed with high variability in AML cells. Growth factor stimulation revealed impaired phosphorylation of S6 protein and 4E-binding protein in mTORlow cells. Upon transfection of these cells with a construct encoding mTOR phosphorylation of S6 protein was restored. Nevertheless, rapamycin treatment resulted in a comparable growth reduction and induction of apoptosis in mTORhigh and mTORlow cells. Down-regulation of mTOR by siRNA, however, reduced cell growth in mTORhigh cells only.

AML cells expressing different levels of mTOR are a useful tool to study the involvement of mTOR in AML cell responses. The finding that rapamycin treatment induces a comparable effect on cell growth in mTORhigh and mTORlow cells raises the question of alternative targets in AML cells. Screening a kinome siRNA library will hopefully help identify novel kinases that modulate the sensitivity of AML cells to rapamycin and uncover new therapeutic targets in this cancer.

5

Label-free quantitative phosphoproteomics reveals that the TORC1 substrate Sch9 is a central regulator of ribosome biogenesis

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The Target of Rapamycin Complex 1 (TORC1) was originally described in yeast but appears to be conserved in all eukaryote cells. This Ser/Thr kinase complex is a central regulator of eukaryote growth, serving to couple environmental cues to the intracellular growth machinery. In addition to stimulating anabolic processes, TORC1 signals also repress catabolic processes, such as macroautophagy, and the transcriptional induction of multiple stress response programs. To better understand how TORC1 signals to these many distal readouts we have developed and employed a novel, label-free, quantitative, phosphoproteomic methodology. Furthermore, genetic approaches allowed us to partition rapamycin-sensitive phosphorylation events amongst Tap42/PP2A and Sch9, two bona fide TORC1 effectors in yeast. The data generated revealed many new aspects of TORC1 signaling, but we chose to probe in more detail how TORC1 promotes ribosome biogenesis. Ribosome biogenesis is probably the most energy-consuming anabolic process of the cell as it requires massive transcription output from all three nuclear RNA polymerases. We now demonstrate that Sch9, a Ser/Thr protein kinase of the AGC family, plays a central role in coupling TORC1 to all transcriptional aspects of ribosome biogenesis. Focusing on how TORC1 influences RNA Polymerase III activity, we found that Sch9 promotes tRNA genes expression by directly phosphorylating and thereby inhibiting Maf1, a conse rved repressor of RNA Polymerase III. We have previously proposed that yeast Sch9 is the functional ortholog of mammalian S6K1. Consistently, S6K1 has been shown to mediate mammalian (m)TORC1 signals to all three nuclear RNA polymerases. Critically, dysregulation of mTORC1, S6K1 and RNA polymerase III have all been associated with cellular transformation. Thus, yeast in general and the elucidation of TORC1-mediated signals in particular, continue to provide important knowledge regarding mammalian tumorigenesis.

6

The soluble form of the cancer associated L1 cell adhesion molecule is a pro-angiogenic factor

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A soluble form of the L1 cell adhesion molecule (sL1) is released from different tumor cells and can be found in serum and ascites fluid of uterine and ovarian carcinoma patients. sL1 is a substrate for different RGD binding integrins and can be deposited in the extracellular matrix. In this study we describe a novel function of this physiologically relevant form of L1 as a pro-angiogenic factor. We demonstrated that the anti-L1 monoclonal antibody (mAb) chCE7 binds to the sixth Ig-like domain of human L1 which contains a single Arg-Gly-Asp (RGD) sequence. Mab chCE7 inhibited the RGD-dependent adhesion of ovarian carcinoma cells to sL1 and inhibited the sL1 induced growth of bovine aortic endothelial (BAE) cells. Furthermore the sL1 induced matrigel invasion and tube formation of BAE cells was inhibited by mAb chCE7. A combination of sL1 with vascular endothelial growth factor-A (VEGF-A165), which is an important angiogenic inducer in tumors, strongly potentiated VEGF receptor-2 tyrosine phosphorylation in BAE cells. Chick chorioallantoic membrane (CAM) assays revealed the pro-angiogenic potency of sL1 in vivo which could be abolished by chCE7. These results indicate an important role of released L1 in tumor angiogenesis and represent a novel function of antibody chCE7 in tumor therapy.

7

Elucidating the potential efficacy of indole derivatives in prevention of cancer cell growth: Development of new strategies for cancer prevention and traetment

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Epidemiological studies have demonstrated a decreased incidence of cancer in humans consuming large amounts of cruciferous vegetables. These vegetables contain glucobrassicin, which hydrolysis by myrosinase to indole-3-carbinoe that converted to 3,3'-diindolylmathane (DIM). These compounds have inhibitory effects on proliferation of human breast, prostate and colorectal cancer cells. These compounds exert their effects in cancer cells through the induction of apoptotic which was induced through the mitochondrial pathway; translocation of cytochrome C from mitochondria to the cytosol and activation of initiator caspase 9 and the effectors caspaces, 3 and 6 leading to poly A ADP-ribose polymerase (PARP) cleavage. Additinally, we have found that DIM increased the expression of NDRG1, a differentiation factor. Treatment with DIM (5 and 10 mg/kg, 3 times a week) caused a significant deceleration in volumes and weights of tumours which were induced in C57BL/6 mice, by transplanting TRAMP-C2 prostate cancer cells subcutaneously. Histopathological studies indicated that DIM induces apoptosis in the tumour cells. Moreover, we have found that treatment with for 5 weeks, significantly prevent tumor development in transplanting animals. Tumors were developed in 80% of control and in 40% of treated animals. Moreover, the tumors developed in treated animals were significantly smaller than that developed in controls. The treatment of DIM was not accompanied by liver and kidney toxicity as it was detected by biochemical tests. Thus, it appears that DIM may offer an effective and non-toxic therapeutic mean against tumour growth, and may serve as a potential natural antitumorogenic compound in humans.

1. Ge X., Yannai S., Gruener N., Renert G. and Fares FA., 3,3'-diindolylmethane Induced apoptosis in human cancer cells. Biochemical and Biophysical Research Communications. 228: 153-158, 1996.
2. Maya Nachshon-Kedmi, Fuad A. Fares, and Shmuel Yannai. Therapeutic Activity of 3,3'- Diindolylmethane on Prostate Cancer in an In Vivo Model. Prostate. 61:153-160, 2004
3. Maya Nachshon, Shmuel Yannai and Fuad A Fares. Induction of apoptosis in human prostate cancer cell line, PC3, by 3,3' - Diindolylmethane through the mitochondrial pathway. British Journal of Cancer 91:1358-1363, 2005

8

A lymphotoxin driven pathway to chronic hepatitis and hepatocellular carcinoma

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Hepatitis B and C viruses (HBV, HCV) are the major cause of chronic hepatitis and hepatocellular carcinoma (HCC), the most common primary liver cancer. Here we report a drastic upregulation of the pro-inflammatory cytokines lymphotoxin (LT) a, b, LIGHT and their receptor (LTbR) in HBV or HCV infected human livers and in HCC. Transgenic mice with hepatocyte-specific overexpression of LTab showed chronic progressive hepatitis followed by HCC development. Removal of IKKb from hepatocytes and ablation of lymphocytes, but not depletion of TNFR1 prevented chronic hepatitis and HCC formation. Acute in vivo LTbR stimulation induced hepatic transcriptional changes similar to LTab-overexpressing livers and depended exclusively on LT-signal transduction within hepatocytes. These results suggest that sustained LT signaling on hepatocytes is causally linked to chronic hepatitis and HCC development. Thus, interference with LTbR signaling may be of therapeutic value in HBV or HCV induced liver diseases.

9

Role of notch signaling in breast epithelial cells

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Notch signaling is known for its oncogenic activity in the mouse mammary gland and lately evidence has accumulated that it may have a similar role in the human breast. However, the mechanism underlying its oncogenic effect and its physiological role in breast epithelial cells are not well established. In order to understand which cellular functions Notch signaling is affecting we activated Notch signaling in primary human breast epithelial cells (HBEC) by ectopically expressing Notch1 intracellular domain (NICD). Increased Notch activity results in inhibition of cell growth, detachment of HBECs from the plate, and formation of multicellular spheroid structures. This phenotype is associated with downregulation of ECM related proteins as well as p63. Coexpression of Delta N-p63 (DNp63) with NICD rescues the NICD -induced phenotype suggesting p63 downregulation as the key mechanism of the notch induced growth arrest and cell detachment. Analysis of the two breast epithelial cell subtypes, the myoepithelial and luminal cells freshly isolated from human breast tissue shows that Notch ligands are expressed in myoepithelial whereas the cognate receptors are enriched in luminal cells, inferring that Notch signaling is activated in luminal cells in vivo. P63 expression, on the other hand, is restricted to the myoepithelial cell compartment, and modulation of p63 interferes with the cell lineage specific markers and phenotype showing that it is functionally important for myoepithelial lineage. This segregation of Notch activity in luminal cells and p63 in myoepithelial cells suggest that inhibition of p63 by Notch signaling could be involved in cell-fate determination/maintenance in the breast epithelium.

10

Evaluation and functional characterization of periostin in lung and kidney cancer

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Periostin, (also termed osteoblast-specific factor 2) (gene name: POSTN), is a secreted protein of 93 kDa and six different splice isoforms exist. It shares homology with the insect cell adhesion molecule fasciclin I and with human beta IgH3 and is induced by TGF beta and BMP-2. Periostin promotes integrin-dependent cell adhesion and motility and supports osteoblastic cell lines attachment and spreading. It is expressed in few normal tissues and is overexpressed in many cancerous tissues (lung, kidney, breast, ovarian, colon, head and neck cancer). High periostin expression levels correlate with tumor aggressivenes (1, 2). Periostin also promotes epithelial mesenchymal transition (EMT) of carcinoma cells which leads to invasion and metastasis (3). Therefore it is a predictive marker and a potential target for tumor therapy. In our previous study POSTN expression was analysed in a tissue microarray of 538 non-small cell lung cancers: POSTN overexpression is associated with increasing TNM stage and grade. This project aims at the analysis of expression and localization of periostin isoforms in lung and kidney cancer and at their functional characterization. Our current study identified four periostin isoforms in the lung and three isoforms in the kidney. Each of the isoforms was coexpressed in tumor and matched non-neoplastic tissue. The cloning analysis of renal periostin isoforms revealed a new isoform which has to date not been reported in other human organs. The western blot analysis of lung and kidney cancer tissue and pleural effusions from lung cancer patients shows the presence of a cleaved form of periostin of 55 kDa.

1. Sasaki H, Dai M, Auclair D, Fukai I, Kiriyama M, Yamakawa Y, et al. Serum level of the periostin, a homologue of an insect cell adhesion molecule, as a prognostic marker in nonsmall cell lung carcinomas. Cancer 2001; 92(4):843-8.
2. Gillan L, Matei D, Fishman DA, Gerbin CS, Karlan BY, Chang DD. Periostin secreted by epithelial ovarian carcinoma is a ligand for alpha(V)beta(3) and alpha(V)beta(5) integrins and promotes cell motility. Cancer Res 2002; 62(18):5358-64.
3. Yan W, Shao R. Transduction of a mesenchyme-specific gene periostin into 293T cells induces cell invasive activity through epithelial-mesenchymal transformation. J Biol Chem 2006; 281(28):19700-8.

11

Investigating the role of PTPRJ in renal cell carcinoma

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The von Hippel-Lindau (VHL) tumor suppressor is a multifunctional protein. VHL mutations are common in sporadic clear cell renal cell carcinoma (ccRCC). Our previous mass spectrometry and qRT-PCR experiments identified surface proteins that were differentially expressed in VHL-positive and -negative ccRCC cell lines. Here we focus on PTPRJ, a gene encoding for a receptor type protein tyrosine phosphatase, which is putatively positively regulated by VHL. Loss of heterozygosity, deletions and missense mutations often occur in PTPRJ gene in cancer [1], PTPRJ has been found absent or downregulated in several human neoplasias [2], and its re-expression in tumor cell lines leads to antitransforming effects and diminished cell mobility and proliferation [3] . Therefore, several lines of evidence suggest that PTPRJ might be a tumor suppressor gene. RNA in situ hybridisation, qRT-PCR and Western blot analysis suggest that PTPRJ expression increases in ccRCC cell lines expressing VHL compared to VHL-negative cell lines. HEK293 cells exposed to the prolyl hydroxylase inhibitor DMOG showed a decrease of PTPRJ mRNA expression with an inverse correlation to HIF-1α. Expression of phosphorylated c-Met, a molecular target of PTPRJ, showed an inverse correlation to VHL and PTPRJ. Furthermore, immunohistochemical experiments localized phospho c-Met to the cell membrane in VHL-expressing ccRCC cell lines, whereas in VHL-negative cell lines, phospho c-Met was also detected in the nucleus. These results suggest a possible role of the VHL/HIF-axis in the expression and regulation of, PTPRJ and phospho-c-Met in kidney cancer.

1. Ruivenkamp CA, van Wezel T, Zanon C, Stassen AP, Vlcek C, Csikos T, et al. Ptprj is a candidate for the mouse colon-cancer susceptibility locus Scc1 and is frequently deleted in human cancers. Nat Genet 2002; 31(3):295-300.
2. Trapasso F, Yendamuri S, Dumon KR, Iuliano R, Cesari R, Feig B, et al. Restoration of receptor-type protein tyrosine phosphatase eta function inhibits human pancreatic carcinoma cell growth in vitro and in vivo. Carcinogenesis 2004; 25(11):2107-14.
3. Balavenkatraman KK, Jandt E, Friedrich K, Kautenburger T, Pool-Zobel BL, Ostman A, et al. DEP-1 protein tyrosine phosphatase inhibits proliferation and migration of colon carcinoma cells and is upregulated by protective nutrients. Oncogene 2006; 25(47):6319-24.

12

Loss of VHL and hypoxia provoke PAX2 upregulation in clear cell renal cell carcinoma

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The paired box gene 2, PAX2, encodes for a transcription factor that is upregulated during nephrogenesis and becomes silenced in mature epithelium of the glomeruli, the proximal and distal tubules. Re-activation of PAX2 has been frequently observed in clear cell Renal Cell Carcinoma (ccRCC), a tumor type characterized by loss of von Hippel-Lindau (VHL) tumor suppressor function. The regulation of PAX2 expression in ccRCC is unknown. Here we show that both loss of pVHL function and hypoxia leads to strong PAX2 re-expression. Using luciferase reporter gene assays no induction was obtained in spite of 6 hypoxia-response element motifs identified in the promoter of PAX2. Comprehensive immunohistochemical analyses of 831 human ccRCC showed significant correlations between PAX2, HIF1a and HIF2a target CCND1 expression patterns. Notably, PAX2 expression was highly associated with early stage, well differentiated ccRCC and, consequently, better clinical outcome. Additional analyses i ndicated that PAX2-repressor WT1 and cancer-linked hypomethylation are not important for transcriptional regulation of PAX2 in ccRCC. We conclude that in ccRCC PAX2 re-activation is driven by HIF-dependent mechanisms following pVHL loss.

13

A recombinant anti-PSMA single-chain immunotoxin as novel therapeutic option for advanced prostate cancer

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Prostate cancer continues to be the second most common cancer among men in industrialized countries, and represents the third leading cause of cancer deaths. Whereas early disease can be successfully treated, no curative therapy exists for advanced stages. Therefore new agents for the management of this tumor are urgently needed. In recent years, the prostate specific membrane antigen (PSMA), a type II membrane glycoprotein, has generated great interest for therapeutic intervention, because it is primarily expressed on the prostate, abundantly expressed on prostate cancer epithelial cells and even upregulated in androgen insensitive and metastatic disease. As a new therapeutic approach, we constructed a recombinant immunotoxin, called A5-PE40, consisting of a single-chain antibody fragment (scFv) against PSMA as the binding domain, and PE40, a truncated form of Pseudomonas Exotoxin A, as the toxin domain. A5-PE40 showed a specific binding to PSMA-positive cells of the androgen-independent human prostate cancer line C4-2. Furthermore, a high and specific cytotoxicity of A5-PE40 against C4-2 cells was measured with an IC50 value of 220 pM. In vivo, immunotoxin treatment of SCID mice bearing C4-2 xenografts caused a significant inhibition of tumor growth, whereas control tumors remained totally unaffected. Due to its high and specific cytotoxicity and antitumorous activity the immunotoxin A5-PE40 represents a promising candidate for the future immunotherapy of advanced prostate cancer.

1. Wolf, P. et al.: Anti-PSMA immunotoxin as novel treatment for prostate cancer? High and specific antitumor activity on human prostate xenograft tumors in SCID mice. Prostate 68, 129-138 (2008).
2. Wolf, P. et al.: A recombinant PSMA-specific single-chain immunotoxin has potent and selective toxicity against prostate cancer cells. Cancer Immunol. Immunother. 55, 1367-1373 (2006).

14

Identifying novel targets of the melanoma-critical gene transcription factor MITF

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Malignant melanoma is a chemotherapy-resistant cancer with high mortality. Recent advances in our understanding of the disease at the molecular level have indicated that it shares many characteristics with developmental precursors to melanocytes, the mature pigment-producing cells of the skin and hair follicles. The development of melanocytes absolutely depends on the action of the microphthalmia-associated transcription factor (MITF). MITF has been shown to regulate a broad variety of genes, whose functions range from pigment production to cell-cycle regulation, migration and survival. However, the existing list of targets is not sufficient to explain the role of MITF in melanocyte development and melanoma progression. DNA microarray analysis of gene expression offers a straightforward approach to identify new target genes, but standard analytical procedures are susceptible to the generation of false positives and require additional experimental steps for validation. Here, w e introduce a new strategy where two DNA microarray-based approaches for identifying transcription factor targets are combined in a cross-validation protocol designed to help control false-positive generation. We use this two-step approach to successfully re-identify thirteen previously recorded targets of MITF-mediated upregulation, as well as 71 novel targets. Many of these new targets have known relevance to pigmentation and melanoma biology, and further emphasize the critical role of MITF in these processes.

Hoek et al., 2008 - Pigment Cell Melanoma Res 21(6):665-676
Hoek et al., 2008 - Cancer Res 68(3):650-656
Hoek, 2007 - Pigment Cell Res 20(6):466-484

15

Internet based health promotion campaigns against skin cancer - Results of the first skincheck in Switzerland in May 2008 -

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Skin cancer compromises the majority of all cancers and shows rising incidence worldwide. Common prevention programs appeal limited population groups, men respond in a lower frequency. To achieve generally unaffected population groups with distinct pre-selection, the internet based health promotion campaign Skincheck as a pilot project was created. Users were educated in risk factors for skin cancer and self examination of the skin. Afterwards, photographs of suspicious skin lesions could be sent for teledermatological evaluation. Those participants were requested afterwards about compliance and dermatological diagnosis.
Skincheck arouse public interest particularly amongst young and males (mean age: 38 years; 53.04% (N=262): male). 141 of the 494 sent lesions (28.54%) were considered as suspicious. Requests via email were answered by 29 females (46.03% of all females) and 46 males (59.74% of all males) and revealed higher female distribution in younger age classes (34.48% of all females between 16-29 years) while higher age classes were mostly represented by males (within 30-49 and 50-64 years each 21.74% of all males). Participants beyond 64 years consisted solely of males (8.7% of all males). Following requests, at least, 8 malignant lesions (8.51%) (1 melanoma in situ, 1 squamous cell carcinoma, 4 basal cell carcinoma among others) and 3 dysplastic melanocytic nevi (3.32%) have been detected in recommended medical referral.
Thus, addition of internet based educational programs to existing health promotion campaigns will enhance participation of the population; internet based tolls might preferentially target young and male population which usually participate in a lower frequency in prevention programs.

1. Dummer R, P.R., Anliker M, Banic A, Braathen LR, Braun R, Bulliard JL, Gerber B, Hohl D, Itin P, Mihic-Probst M, Moser M, vanBelow G, Überraschende Ergebnisse der 1. Hautkrebswoche in der Schweiz, 15. -19. Mai 2006, Krebsliga Schweiz, Bundesamt für Gesundheit, Schweizerische Gesellschaft für Dermatologie und Venerologie.

2. Dummer, R. and T. Hofer, Hautkrebswoche 2008. Dermatologica Helvetica, in press 2009 3. Bulliard, J.L., et al., Prevention of cutaneous melanoma: an epidemiological evaluation of the Swiss campaign. Rev Epidemiol Sante Publique, 1992. 40(6): p. 431-8.

16

NDR kinases function as tumor suppressors in vivo

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The recently described Hippo-tumor suppressor pathway in flies is also emerging in mammalian systems. The major players of this pathway in flies are the Ste20 like kinase Hippo, the co-activator/scaffold proteins Salvador and Mats, the NDR kinase Warts/Lats and the transcriptional coactivator Yorkie. This pathway acts as tumor suppressor pathway by negatively regulating the Yorkie proto-oncogene. Although it has been described that in flies Hippo is able to regulate both Warts and the other NDR kinase family member Tricornered, it has not been established, whether Tricornered also functions as a tumor suppressor in invertebrates.
Recent work in our group established the Hippo homologue MST1 as upstream kinase for NDR1/2 in apoptosis induction and the regulation of centrosome duplication. Both processes have been shown to be linked with tumor development, but so far experimental evidence was lacking connecting loss of mammalian NDR1/2 to the development of tumors.
Here we demonstrate, that complete or partial loss of NDR1 predisposes mice to the development of T-cell lymphoma, although the deficiency of NDR1 is initially compensated by the other NDR isoform NDR2. We show that in tumors the protein level of both isoforms are reduced suggesting a tumor suppressive function of NDR1/2. In addition we found, that in a subset of human T-cell lymphoma samples NDR levels are reduced indicating a tumor suppressive role for NDR kinases also in humans. For the first time we present data indicating a tumor suppressive function for NDR kinases in vivo.

17

A dual role of activin in skin carcinogenesis

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Activin is a member of the transforming growth factor-beta; family of growth and differentiation factors, which regulate a wide variety of biological processes. Recent studies suggest a role of activin in the pathogenesis of different types of cancer. Interestingly, depending on the experimental system used, activin exhibited either pro- or anti-tumorigenic effects. In our laboratory important roles of activin in skin homeostasis and wound repair have been demonstrated. Moreover, expression of activin A was found to be strongly up-regulated in human epidermal skin cancer as compared to normal skin. Since it is widely accepted now that there are many similarities between wound healing and cancer, we wondered if activin could play a role in skin cancer.
To investigate the role of activin in a mouse model of skin cancer we subjected mice expressing activin A under the control of keratin 14 promoter to the two-stage chemical skin carcinogenesis protocol. Interestingly, activin overexpression in keratinocytes dramatically increased the susceptibility to chemically-induced skin tumor formation. Surprisingly, mating of activin-overexpressing mice with transgenic mice expressing a dominant-negative mutant of the activin receptor IB in keratinocytes did not rescue the pro-tumorigenic phenotype, but even aggravated it. This indicates that activin has a dual function in skin cancer: it promotes tumor growth indirectly via activation of stromal cells, and it has a direct tumor-suppressive effect via keratinocytes. The mechanisms of these activin actions we are currently investigating.

1. Munz B, Smola H, Engelhardt F, et al. Overexpression of activin A in the skin of transgenic mice reveals new activities of activin in epidermal morphogenesis, dermal fibrosis and wound repair. Embo J 1999;18:5205-5215.
2. Werner S, Alzheimer C. Roles of activin in tissue repair, fibrosis, and inflammatory disease. Cytokine Growth Factor Rev 2006;17:157-171.
3. Schafer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol 2008; 9:628-638.

18

The G-allele of MTR c.2756A>G is associated with different highly proliferative brain tumors: an effect of global DNA methylation?

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In case-control, monocenter association studies we investigated 328 consecutive glioblastoma multiforme (GBM) patients, 290 meningioma patients, and 31 primary central nervous system lymphoma (PCNSL) patients of Caucasian origin recruited for the brain tumor library of the University Hospital of Bonn between September 1994 and May 2006. Different populations of age- and gender-matched, apparently healthy, white Caucasian area residents without a history of cancer served as controls.
We performed genotyping of the polymorphisms 5,10-methylenetetrahydrofolate reductase (MTHFR) c.677C>T and c.1298A>C, 5-methyltetrahydrofolate-homocysteine S-methyltransferase (MTR) c.2756A>G, reduced folate carrier 1 (RFC1) c.80G>A, cystathionine beta-synthase (CBS) c.833T>C and 844ins68, transcobalamin 2 (Tc2) c.776C>G and dihydrofolate reductase (DHFR) c.594+59del19.
In GBM, PCNSL and meningioma WHO grade III patients the G-allele of MTR c.2756A>G was significantly under-represented (GBM: $p<0.001$;PCNSL: $p<0.005$;meningioma WHO III: $p<0.001$), suggesting a protective effect of the G-allele in these highly proliferative brain tumors.
In a different population including 313 individuals we investigated leukocyte global DNA methylation status using methylation-sensitive enzymatic digestion followed by a single nucleotide extension assay and MTR c.2756 A>G genotype. The MTR genotype was significantly associated with global DNA methylation showing an allele-dosage effect (expressed as % methylated CpG): MTR c.2756A>G, AA: 41.3±14.9; AG: 36.4±18.2; GG:30.8±16.9 ($F=5.55$; $df=2$; $p=0.005$).
Therefore, the influence of the MTR variant on DNA methylation may explain the association with the different brain tumors. Manipulation of methionine metabolism by diet might be an interesting candidate for brain tumor prevention.

19

HNF3a is overexpressed in metastases and hormone refractory prostate cancer and is associated with aggressive disease

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Aims. In previous studies we have identified hepatocyte nuclear factor-3a (HNF-3a) as overexpressed in prostate carcinoma on mRNA level. The clinical significance of HNF3a protein expression is still unknown.
Methods: HNF3a protein expression was analysed immunohistochemically in normal prostate tissue (n=40), primary prostate cancer (n=337), metastases (n=39), hormone refractory prostate cancer (HRPC, n=26).

Results. Strong HNF3a overexpression has been detected in 55% of normal tissues, whereas 64% of primary carcinomas, 72% of metastasis and 88% of HRPC showed a strong nuclear HNF3a staining. In primary tumours, HNF3a expression correlated significantly to higher Gleason Scores but not to pT-category or R-status. Progression free survival times were shorter in HNF3a overexpressing cases, although this failed significance.

Conclusions. This study confirms the overexpression of HNF3a in prostate cancer and demonstrates an increasing frequency of HNF3a overexpression during tumor progression as well as in dedifferentiation. However HNF3a fails to represent an independent prognostic marker. The particularly high levels in HRPC merits further analysis.

20

Improving QOL in breast cancer patients in resource poor developing nations: Supportive care efforts by an Non-Govt-Organisation [NGO]

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Issues. Social-stigma/Fatigue/sexual-dysfunction/Sleeplessness/depression, pain common in Breast-cancer-sufferers. Palliative-care inaccessible in rural/tribal India. Hence our NGO took initiatives since October 2005.
Objective: 53/year Indian-women die from breast-cancer. statistically >90% express sexual-dysfunction, 68% unbearable-pain; 70% social-neglect/humiliation ; 54% sleeplessness, nausea/vomiting; 37% fatigue and 64% depression. Importance of spirituality/religion in coping with terminal-illness is increasingly recognized, Hence Our NGO-nurses followed-up poor rural women unable to afford Rx & needed of palliative-care. we involved community-leaders to make more women involved in our spiritual-healing-sessions.
Methods. surveyed 55 breast-cancer-cases through QOL-questionnaires. After 6 sessions of Counseling/palliative-support QOL improved to statistically significant. symptom assessment performed on weekly-basis. Traditional faith-healers involved for more psychological-impact on patients.
Results. USED opioids in 35%. Diazepam 23% patients. Pethidine 56%, tramadol 22%. >30% cases in advanced-stage. 20 specialist palliative-care beds required for Rural/tribal population of 6,00,000. 53% expressed religious/community support/faith was important factor to cope with breast-cancer. significant correlations between higher scores of spirituality with absence of depression. Higher-scores of QOL (ANOVA $p < 0.001$) correlated with lack of sexual dysfunction/pain. Our NGO-initiative suggests that over 70% patients will need well trained specialist for home-based-care unit.
Conclusions. Life-span/QOL of breast cancer-sufferers depends on social acceptance & appropriate-palliative-care. NGO-personals should be trained in Palliative-care-services. These data is being used for palliative care advocacy. Spiritual-well-being increases end-of-life despair in terminally-ill. Field of Spiritual/psycho-social/community support is fertile ground for further investigations.

We share our NGO-project experiences/difficulties with participants of 9th Charles Rodolphe Brupbacher Symposium 2009.

21

Cost-availability analysis of breast cancer treatment : NGO's can improve access to cancer chemotherapy resource poor nations

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Issues. National-cancer-registry based data demonstrates subsidized Psychosocial support/HRT & treatment-availability are major issues for Breast-cancer-sufferers in resource-poor-nations. Hence our Non-Govt-Organisation analysed & started this public health-policy recommendation.
Objectives. NGO's close to rural/tribal-communities. Cost of running NGO economical than medical-institution. Anti-cancer-drugs cost prohibitive. Govt-Health-Depts need to workout strategy to increase chemotherapy-access. In resource-poor-setting unaffordable cost leads to poor-therapeutic-compliance therefore high mortality. We develop training program to develop of sound /sustainable cancer-care for rural communities
Methods. we have 28NGO-volunteers. No national-program for financial help to cancer patients. In breast-cancer-sufferers Individual's sexual-identity, sexual-function dramatically wounded. Women suffer silently physically/emotionally. cancer-Care programs designed towards rural/tribal population needed. Our NGO since one year offers guidance for Rx-funding, guidance those going to city-oncology-centres. This project is unique as we are training farmers & village leaders to develop a peer-peer model. Depending on support given by donors we give poor people little-financial-assistance for chemo-radiotherapy & improve access to governmental hospitals, we started with two towns & intend to help 12 villages by 2010.
Results. We did face hiccups in mobilising volunteers/resources. This strategy has minimum maintenance-cost & high-acceptability. Forums like 9th Charles-Rodolphe-Brupbacher-symposim 2009. should help NGO-activists need work with European researchers to form workgroup to develop this concept.
Conclusions. Economical-factors/access to therapy changes out-come of Breast-cancer-Rx. With little training our community NGO in rural/tribal India formed well knit volunteers-group who is giving free part-time dedicated service. HRT/psychosocial-support improves QOL reduceing difficulties faces by resource-poor-southern-countries. We urge 2009-Zurich-symposium participants to share views/expertise on this burning-issue.

22

Targeting phosphoinositide 3-kinases as a new potential therapeutic strategy for the treatment of medulloblastoma

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Medulloblastoma is the most common malignant brain tumor in childhood and represents the main cause of cancer-related death in this age group. The phosphoinositol 3-kinase (PI3K) pathway, targeted by different genetic alterations in many human cancers, has been shown to play an important role in the regulation of cell survival and proliferation in medulloblastoma. Analysis of the expression pattern of the class IA PI3K isoforms demonstrated that the catalytic isoform p110alpha is overexpressed in medulloblastoma cell lines and tumor samples. Effects on cell survival and downstream signalling were analysed following downregulation of p110alpha in medulloblastoma cells by means of RNA interference. Downregulation of the expression of p110alpha in DAOY cells by RNAi resulted in a decrease in cell growth and cell proliferation. To gain further insight into the downstream pathways mediating p110alpha signals, we performed a DNA microarray analysis. Here, we screened the changes in the expression of DAOY cells caused by RNAi-mediated downregulation of p110alpha on the Affymetrix Gene Chip HG U133 Plus 2. We identified a group of genes involved in important cellular events, such as cell growth and apoptosis, to be affected by the downregulation of p110alpha. The expression of AKT2, CDK6 and BCL6 was up-regulated upon PIK3CA silencing, while IGF1R and LIFR were down-regulated. These genes might be important factors mediating p110alpha's signals in medulloblastoma cell responses and represent new interesting target molecules for further studies.

Ana S. Guerreiro, Sarah Fattet, Barbara Fischer, Tarek Shalaby, Shaun P. Jackson, Simone M. Schoenwaelder, Michael A. Grotzer, Olivier Delattre and Alexandre Arcaro (2008) Targeting the PI3K p110α Isoform Inhibits Medulloblastoma Proliferation, Chemoresistance, and Migration.
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23

Functional relevance of PI3KC2beta tyrosine phosphorylation in the activation and regulation of the enzyme

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Phosphoinositide 3-kinases (PI3Ks) are extremely important for cell proliferation, differentiation and motility processes which if aberrant are implicated in cancer. Compared to class I and class III PI3Ks little is known about the functions of class II PI3Ks in human cancer. Recent studies have documented a role for PI3KC2beta in cell migration and survival of human tumour cells by distinct molecular mechanisms. A better understading of the mechanisms of activation and regulation of these enzymes is therefore very important. In this study, we wanted to characterize the functional relevance of 4 newly identified Tyr phosphorylation sites (Y68, Y127, Y228, Y1541) in the class II PI3KC2beta for the activation and regulation of the enzyme in context of cell migration, proliferation and protection from anoikis. The phosphorylation status of the various Tyr to Phe PI3KC2beta mutants was investiagted in HEK293 and A-431 cell lines. Additionally we used HT29 colon cancer cells where two (Y127, Y228) out of four Tyr phosphorylation sites were initially characterised. In view of the ability of Src family tyrosine kinases to phosphorylate PI3KC2beta we also used these mutants to identify which of the Tyr phosphorylation sites are Src family kinase targets. Together these studies will hopefully lead to a better understanding of the mechanism of PI3KC2beta regulation and its possible involvement in human cancer.

24

Targeting PI 3-kinase isoforms in human glioblastoma

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Phosphoinositide 3-kinases (PI3K), a class of lipid kinases, lie downstream of several different growth factor receptors and play a crucial role in controlling a wide variety of intracellular signaling events. Mutations in the tumor suppressor gene phosphatase and tensin homologue deleted on chromosome 10 (PTEN) are frequently observed in glioblastoma (GB), highlighting the importance of the PI3K signaling pathway in this context. Additionally, activating mutations in the PIK3CA gene (encoding class IA p110alpha isoform) occur in a broad range of solid tumors, including GB. This study aims at further elucidating the role of the PI3K isoforms p110alpha, p110beta and p110delta (class IA) in the context of cell proliferation, survival, and Akt signaling pathway activation in human GB.
Individual PI3K isoforms were inhibited by using isoform-specific pharmaceutical inhibitors or siRNA and the effect on cell viability, downstream signaling and sensitivity to chemotherapeutic agents were analyzed.
Only p110alpha inhibitor PIK75 showed a significant effect on GB cell viability, both in cell lines and ex vivo cultures. Furthermore, targeting p110alpha impaired anchorage-independent growth of GB cells. Inhibition of p110beta or p110delta did not result in similar effects on GB cell responses. Additionally, combinational treatment of p110alpha isoform-specific inhibitor with chemotherapeutic agents led to a sensitization of cell lines and ex vivo cultures to chemotherapy. These responses correlated with an inhibition of Akt activation upon treatment of GB cells with p110alpha inhibitors.
The studies outlined here should lead to new insights into the selective functions of PI3K isoforms and their downstream signaling targets in human glioblastoma biology and facilitate the validation of targeted drug therapies to cure this common type of malignant brain tumor.

25

Immunosuppression affects CD4+ mRNA and induces Th2 dominance in the inflammatory microenvironment of cutaneous squamous cell carcinoma in organ transplant recipients

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Squamous cell carcinoma (SCC) represents the most frequent tumor associated with drug-induced immunosuppression in organ transplant recipients (OTRs). Compared to general population, OTRs are at up to 100-fold risk of SCC development. The immune system plays a major role in the fight against SCC, however little is known about the local inflammatory response in SCC. We analyzed quantity and quality of the peritumoral inflammatory SCC microenvironment in immunocompetent patients and OTRs.
SCCs from 15 immunocompetent patients and 13 OTRs were analyzed by RT-PCR for CD4, CD8, TBET, GATA-3, FOXP3, RORC, IFN-gamma, IL-4, TGF-beta, IL-10 and IL-17A mRNA expression. CD3, 4, 8 and FOX-P3 protein expression was studied by immunohistochemistry.
Considerable inflammation was seen in both groups. CD4+ mRNA was diminished in immunosuppression, while CD8+ did not vary. T-BET mRNA expression did not vary between the groups; however mRNA expression of INF-gamma was significantly decreased with immunosuppression. Although GATA-3 mRNA was markedly increased in OTRs, IL-4 expression did not differ significantly. RORC mRNA was significantly increased in OTR group, but IL-17A mRNA level was decreased in immunosupression. FOX-P3 mRNA level was unchanged. IL-10 mRNA expression did not vary between the groups, while TGF-beta was decreased with immunosuppression.
The inflammatory microenvironment of cutaneous SCC in OTRs is characterized by an attenuated state of local immune response comprising a lesser quantity of helper T cell response and a Th2 polarized quality. The dramatically increased cutaneous carcinogenesis and its more aggressive course in OTRs may be influenced by decreased regulatory T cells response.

26

Her2/neu and GRB7 signalling in adeno- and squamous cell carcinoma of the esophagus

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Aims. To investigate protein expression of Her2/neu and the adaptor protein "growth factor bound protein 7" (GRB7) in Barretts' adeno- (BAC) and squamous cell (SCC) esophageal cancers in situ and to functionally interfere with GRB7 signalling to investigate downstream signalling in vitro.
Materials and Methods. Archival tissue samples of 117 esophageal cancers (BAC: n=64, SCC: n=53) were examined for Her2/neu and GRB7 protein expression by semi-quantitative immunohistochemistry. Four esophageal cancer cell lines (BAC: OE19, OE33, SCC: OE21, Kyse410) were analyzed for Her2/neu and GRB7 at DNA (Q-PCR, FISH), mRNA (Q-RT-PCR) and protein (immunofluorescence) levels. In two cell lines (OE19, Kyse 410) GRB7 was inhibited by siRNA and potential downstream targets (P-AKT, Survivin, Cyclin-D1) examined by western blot analysis.
Results. In situ, Her2/neu was strongly correlated with GRB7 expression in esophageal cancers (p=0.0162), but this correlation was specific for BACs (p=0.0209) and not SCCs (p=0.816). Furthermore, Her2/neu and GRB7 were amplified and strongly expressed at the mRNA and protein level in both BAC (OE19, OE33), one SCC (Kyse410), but not in the SCC OE21 cell lines. Down-regulation of GRB7 by specific siRNA resulted in concomitant down-regulation of P-AKT and Survivin, but not Cyclin-D1 in OE19 and Kyse410 cell line.
Conclusion. Her2/neu and GRB7 signalling are a central feature of BACs and GRB7 phosphorylates AKT and induces Survivin. Although also a minority of SCCs or cell lines derived thereof may express this phenotype, Her2/neu GRB7 signalling may indicate a mechanism of resistance to apoptosis of preferentially Barretts adenocarcinoma cells.

27

Complete In vitro reconstitution of base excision repair over 8-oxo-G by human enzymes

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We have recently shown that proliferating cell nuclear antigen (PCNA) and replication protein A (RP-A) allow the correct incorporation of dCTP by DNA polymerase lambda (pol lambda) opposite an 8-oxo-G lesion 1200- fold more efficiently than the incorrect dATP (1, 2). Here we reconstituted in vitro base excision repair of 8-oxo-G lesion. A novel 8-oxo-G specificity assay allowed us to precisely detect and quantify dATP versus dCTP incorporation opposite 8-oxo-G. We show that the replicative pol delta is preferentially incorporating the incorrect dATP opposite 8-oxo-G. The human MutY glycosylase homologue (MutYH) and the apurinic endonuclease 1 (APE1) remove the wrong Adenine opposite 8-oxo-G and cut the abasic site. Subsequently, PCNA and RP-A specifically allow pol lambda, but not pol beta, to faithfully incorporate dCTP onto 8-oxo-G. Finally, DNA ligase I ligates more efficiently the correct dC:8-oxo-G paired product of pol lambda reaction, but in case of pol beta ligat es more efficiently the product with incorrect dA:8-oxo-G pairing. Our data support the idea that pol lambda is the repair enzyme that copes with the oxidative damage in the cell and therefore prevents deleterious G-C to T-A transversion mutations, thus counteracting genetic instability.

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1. Maga, G., Villani, G., Crespan, E., Wimmer, U., Ferrari, E. Bertocci, B. and Hübscher, U.: Nature, 447, 606-609, 2007.

2. Maga, G., Crespan, E., Wimmer, U., van Loon, B., Amoroso, A., Mondello, C., Ferrari, E., Belgiovine, C., Villani, G., Hübscher, U.: Proc Natl Acad Sci U S A. 2008 Dec 30;105(52):20689-94. Epub 2008 Dec 22.

28

Toll-like receptor 9 (TLR9)-induced suppression of Epstein-Barr virus (EBV) lytic gene expression is MyD88 dependent yet independent of NF-kB activation

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Latent EBV exhibits a unique human B-cell transformation capacity. We have demonstrated that immune activation via TLR9 suppresses initiation of EBV lytic replication in acutely and chronically infected human B-cells. Here, we characterized the underlying mechanism in the EBV-infected Burkitt's lymphoma (BL) cell line Akata. Inhibitors of TLR9 activation showed that suppression of EBV switching to lytic gene expression is dependent on functional TLR9 signaling. Moreover, by generating a dominant negative mutant form of MyD88, the adaptor protein for TLR9, we could show that MyD88 is involved in the TLR9-induced suppression of EBV lytic gene expression. Although we provide evidence that the known signaling elements downstream of MyD88 are activated, they do not account for the suppression of BZLF1, the master regulator of EBV lytic gene expression. Surprisingly and contrary to the reported crucial role of the cellular transcription factor NF-kB in TLR9 signaling and regulation of EBV gene expression, using various inhibitors of NF-kB we show that the activation of NF-kB upon TLR9 triggering is not fully responsible for the TLR9-mediated suppression.
We propose that the suppression of EBV lytic replication via TLR9 triggering enhances the number and increases the survival of latently EBV-infected B-cells with transformation potential. This is of particular importance given that malaria which is epidemiologically linked to endemic BL is a strong TLR9 activator.

29

Targeting receptor tyrosine kinase signalling in neuroblastoma

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Receptor tyrosine kinase (RTK) signalling is known to play a crucial role in the development of human neuroblastoma (NB). Downstream of RTKs, the PI3K/Akt/mTOR pathway controls cell responses such as cell proliferation, survival, and motility and is frequently activated in human cancer. We investigated the potential of targeting RTK signalling in neuroblastoma, which is the most common extracranial solid tumour in childhood causing 15% of cancer-related mortality in children younger than 5 years. In order to control metastasis and chemoresistance of NB, new targets for the development of drug therapies are urgently required.
By targeting the RTK signalling pathways with different specific pharmacological inhibitors in a panel of 9 NB cell lines, we could observe a strongly decreased survival of the tumour cells. In particular, NB cells treated with the inhibitors Dasatinib (Abl/Src), NVP-AEE788 (EGFR/ErbB2), and RAD001 (mTOR) showed a significantly reduced viability in vitro. Furthermore the inhibitor of the PI3K isoform p110alpha PIK75 completely inhibited the survival of NB cells at low nanomolar concentrations. To complement these studies, the impact of the inhibitors on apoptosis induction as single agents and in combination with chemotherapy was investigated. The panel of NB cell lines was also screened for expression of the relevant RTKs and signalling molecules by Western blot analysis.
Together, our studies will hopefully lead to a better understanding of the biological function of RTK signalling in controlling cell responses in human NB. Furthermore, this knowledge could point out novel targets and contribute to the development of immediately required new therapies.

30

Transvidin: Delivering biotinylated proteins across cellular membranes

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The delivery of chemical compounds into cells is an important problem that has to be solved during the development of diagnostic tools or pharmaceutical compounds acting on intracellular targets. Often a compound cannot penetrate the cell membrane due to its biophysical properties. Protein transduction domains (PTDs), for instance derived from the HIV-1 TAT protein, can promote the efficient uptake of fused cargo molecules across cellular membranes by an endocytosis-like process. Since PTD penetrate the epidermis and dermis, a system where PTD-mediated penetration and function of the cargo are separated would be a major improvement in terms of drug safety. Our strategy to find a generally applicable solution to this problem utilized cell penetrating streptavidin (SA) derivatives as carriers. Since SA binds biotin, these carriers should, in principle, function with every cargo that is biotinylated. Here, we characterized four different PTD-SA fusion proteins (TAT13-, ANT7-, R9 - and TLM12-SA), which we named Transvidins, as universal transport systems for biotinylated cargos. As a result of the cell-permeable properties of the PTDs, TAT13-, ANT7- and R9-SA were successfully internalized into cultured HeLa cells. PTD-SA proteins were cytosolicly solubilized or appeared in a punctuated pattern around the nucleus which was identified as endosomal or endosomal-like compartments. The concomitant penetration of bound HRP-biotin as a model cargo demonstrated the potential of TAT13- and R9-SA to serve as universal delivery systems for biotinylated cargos. The ultimate goal is the further development of this system for the intracellular delivery of peptide- and protein-based therapeutic agents in vivo.

Gump JM and Dowdy SF. TAT transduction: the molecular mechanism and therapeutic prospects. Trends Mol Med, 2007. 13(10): p.443-8.

31

Anti-proliferative effect of the myc inhibitor NBT-272 in embryonal tumor-derived cell lines

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Embryonal tumors (ET) account for approximately 30% of malignancies in children. MYC family genes are frequently over-expressed in cancer, including in tumors of embryonal origin. The effect of NBT-272, a synthetic quassinoid analogue, was evaluated for its anti-proliferative and cytostatic potential in ET. We observed a drastic reduction of c-Myc protein level in MB-derived D341 cells. A similar effect was observed on MYCN in NB-derived cell lines. A panel of 16 ET-derived cell lines expressing either c-Myc or MYCN were highly sensitive to nanomolar doses of NBT-272, based on a proliferation assays (MTS), as well as on colony formation experiments performed under anchorage-independent conditions. In particular, we extended our previous findings in medulloblastoma to neuroblastoma, Ewing's sarcoma, retinoblastoma, Wilm's tumor and malignant rhabdoid tumor cell lines. The use of a myc-deficient cell line supported the selectivity of the NBT-272 for Myc. The expression of a gro up of 84 apoptosis-related genes was evaluated by qRT-PCR upon treatment of MB cells with NBT-272. A group of apoptosis-related genes were found to be regulated by treatment with NBT-272. TNF-α was found selectively up-regulated in CMYC-transfected DAOY cells following c-Myc inhibition by either NBT-272 treatment or siRNA transfection. Overexpression of c-Myc in DAOY cells sensitized the cells to TNF-α-mediated apoptosis, compared to parental DAOY cells. Together our data show that targeting MYC family genes may represent a novel approach to develop drug therapies for ET.

Knoepfler et al, Myc goes global: new tricks for an old oncogene. Cancer Res. 2007

von Büren et al, Anti-proliferative activity of the quassinoid NBT-272 in childhood medulloblastoma cells. BMCC 2007

Adhikary et al, Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol. 2005

32

TLR mRNA expression patterns in normal and malignant B-cells from different differentiation stages

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Toll-like receptors (TLRs) are key receptors of the innate immune response and show cell-subset specific expression. We hypothesized that expression and function of TLRs are tailored to distinct stages of B-cell development and that these patterns are retained during malignant transformation. We investigated the mRNA expression of TLR genes in hematopoietic stem cells (HSC), normal naïve and memory B-cells isolated from tonsils, and in malignancies originating from immature and mature B-cells from distinct developmental stages.. HSC and plasma cells were unique by their unrestricted expression of TLR1-TLR9, but absence of TLR10. Triggering plasma cells with TLR ligands augmented immunoglobulin production. By contrast, naïve and memory B-cells lacked TLR3, TLR4 and TLR8 but expressed all other TLRs. Given the property of TLR1-TLR9 to augment immunoglobulin production in plasma cells upon pathogen recognition, the presence of TLR10 on B-cells rather than plasma cells and HSC and the lack of a TLR10 agonist may point to a unique function of TLR10. Furthermore, malignant B cells at distinct developmental stages retained the TLR expression profiles of their healthy counterparts. These results are of considerable interest in view that TLR agonists are increasingly used in cancer treatment, and they may have a detrimental rather than a beneficial effect.

33

The wnt/b-catenin signalling pathway cooperates with MDR1 gene-encoded P-glycoprotein in the development of chemoresistance in neuroblastoma cells

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The development of chemoresistance represents a major obstacle in the successful treatment of many cancers. Neuroblastoma (NB) is the most frequent extracranial paediatric solid tumour, and a particularly heterogeneous and devastating disease. To address the mechanisms underlying the chemoresistant phenotype in NB, we analysed the gene expression profile of doxorubicin-resistant cells compared to the sensitive parental cells and identified only 16 up-regulated genes. Not surprisingly the MDR1 gene was included, while the highest overexpressed transcript in 2 chemoresistant cell lines was the frizzled-1 Wnt receptor (FZD1) gene, an essential component of the wnt/b-catenin pathway. We were able to associate the FZD1 up-regulation with a sustained activation of the Wnt/b-catenin pathway by showing nuclear b-catenin translocation and target genes (Cyclin-D1 and IGF2) transactivation. Of particular interest was the specific shRNAmir-mediated FZD1 silencing which induced a parallel strong decrease in MDR1 expression, another recognized b-catenin target gene, revealing FZD1 as a regulator of MDR1 expression through b-catenin activation. Moreover, the significant restoration of drug sensitivity in FZD1-silenced cells, confirmed the FZD1-associated chemoresistance. The analysis of paired RNA samples from 21 patients tumours removed at diagnosis and after chemotherapy revealed a highly significant overexpression of FZD1 and/or MDR1 in samples from relapsed patients, thus underlining the role of FZD1-mediated Wnt/b-catenin pathway activation in the development of clinical chemoresistance.
This report which represents the first implication of the FZD1 Wnt receptor in the chemoresistant behaviour of NB could lead to the identification of new targets to treat aggressive and resistant NB.

34

The expression patterns of IL-1beta in malignant and benign prostate tissue coincide with inhibitory effect of this cytokine on carcinoma cells.

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Although overproduction of IL-1beta is often associated with aggressive growth of many tumors (1), data on its expression in malignant and benign prostate tissue are limited (2,3). The analysis of IL-1beta immune-reactivity in archived carcinoma (N=28) and age-matched benign hyperplastic specimens (BPH, N=30) revealed that in benign areas of tumor samples and BPH specimens, this cytokine was expressed solely by stromal elements, whereas malignant lesions exhibited both stromal and glandular immune-reactivity. We also detected a positive correlation between stromal and glandular IL-1beta expression indexes in the cancer foci. An overall IL-1beta immune-reactivity was significantly lower in neoplastic tissue relative to non-neoplastic tissue and negatively correlated with Gleason's scores of the tumor samples, implying that IL-1beta exerts an inhibitory effect on prostate cancer cells. Indeed, the recombinant IL-1beta attenuated in vitro growth and migration of androgen-dependent LNCaP (but not androgen-independent DU145) carcinoma cells and rendered them susceptible to apoptosis initiated by caspases 2 and 8. This was accompanied by mitochondrial accumulation of pro-apoptotic Bax and Bak proteins and by increase of cytochrome C in the cytosol. Apoptosis-sensitizing activity of IL-1beta was associated with its ability to trigger the secretion of endogenous IL-1beta by carcinoma cells. In contrast, IL-1beta failed to induce its own production by non-neoplastic prostate epithelial cells (PWR-1E) and to affect their migration and survival capacities. Taken together, the results of our immunohistochemical survey and in vitro data indicate that IL-1beta expression patterns in neoplastic and benign prostate tissue coincide with its attenuating effects on malignant properties of carcinoma cells.

(1) Apte RN, Dotan S, Elkabets M, White MR, Reich E, Carmi Y, Song X, Dvozkin T, Krelin Y and Voronov E. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. Cancer Metastasis Rev. 2006 Sep;25(3):387-408.

(2) Ricote M, García-Tuñón I, Bethencourt FR, Fraile B, Paniagua R and Royuela M. Interleukin-1 (IL-1alpha and IL-1beta) and its receptors (IL-1RI, IL-1-RII, and IL-1Ra) in prostate carcinoma. Cancer. 2004 Apr 1;100(7):1388-96.

(3) Bouraoui Y, Ricote M, García-Tuñón I, Rodriguez-Berriguete G, Touffehi M, Rais NB, Fraile B, Paniagua R, Oueslati R and Royuela M. Pro-inflammatory cytokines and prostate-specific antigen in hyperplasia and human prostate cancer. Cancer Detect Prev. 2008;32(1):23-32.

35

CT-antigens in non-melanoma skin cancer

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According to the WHO, non-melanoma skin cancer (NMSC) is the most common cancer world-wide. About 2-3 million new cases of squamous (SCC) plus basal cell (BCC) carcinoma occur globally each year. Despite their comparatively benign nature and low metastatic potential, the standard surgical treatment of SCC and BCC often causes severe pain and disfiguration for the patient.
The risk for NMSC increases significantly in an immunosuppressed state, suggesting a critical role of the immune system for the control of these tumors, however, studies explicitly addressing this are lacking. To investigate whether immunotherapy may be a promising therapeutic modality in NMSC, we embarked to characterize the local and peripheral immune response to cancer-testis (CT) antigens. CT-antigens belong to a large family of antigens that are expressed by a large variety of malignancies and are absent from healthy tissue except for testis and placenta. In addition, cancer patients often develop spontaneous immune responses towards CT-antigens, which illustrates their immunogenicity. Here we present the first extensive study of CT-antigen expression in NMSC. Expression of 23 CT-antigens was investigated in 100 patients via RT-PCR, the expression confirmed by immunohistochemistry on paraffin sections.
Over 50% of the patients expressed at least one CT-antigen; most CT-antigens were co-expressed in groups of up to eleven antigens.
The expression of the antigens will further be correlated to antibody responses and the existence of antigen specific cytotoxic T-lymphocytes in the patient.

36

Melanoma cells remember their origins -a case report

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Human primary and metastatic melanoma lesions frequently show heterogeneous staining pattern for melanocytic marker genes (e.g. Tyr, Melan-A). Cell cultures derived from metastases also show variable expression for these markers, and microarray analyses reveal two major expression signatures present in melanoma cell line libraries. One signature (proliferative) shows up-regulation of genes involved in melanocytic differentiation, while the other (invasive) shows up-regulation of factors involved in modifying the extracellular environment. These expression profiles correlate with phenotypic characteristics of proliferation, invasivness and growth factor sensitivity. We believe that expression of the melanocytic master-regulator Mitf is critical to the phenotype. Indeed, interruption of Mitf expression via siRNA reduceds growth factor susceptibility and proliferation rates; characteristic of the invasive phenotype. We have derived a model in which melanoma cells may switch betw een proliferation and invasion to drive disease progression. Here we interpret a clinical case (thick primary and metastasis to the gall bladder) in the context of our phenotype switching model. Both primary and metastatic lesions show heterogeniety of staining for melanocytic markers, an aspect explained by our model. However, we also show evidence for microenvironment-dependent behaviour in the metastasis which demonstrates that programs reminiscent of healthy melanocytes may yet be recalled by melanoma cells. Melanoma cells encountering basal membrane structures in distal locations seek isolation from their peers and re-express dendrite structures, in vivo behaviours which they are thought to have been lost during progression.

1. Hoek KS, Eichhoff OM, Schlegel NC, Dobbeling U, Kobert N, Schaerer L, Hemmi S, Dummer R (2008) In vivo switching of human melanoma cells between proliferative and invasive states. Cancer Res 68(3): 650-656
2. Hoek KS, Schlegel NC, Brafford P, Sucker A, Ugurel S, Kumar R, Weber BL, Nathanson KL, Phillips DJ, Herlyn M, Schadendorf D, Dummer R (2006) Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. Pigment Cell Res 19(4): 290-8302

37

Identification of cells with stem cell/self renewal properties in malignant pleural mesothelioma

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Mesothelioma (MPM) tumorigenesis is associated with asbestos fibres in the pleural space causing a chronic tissue repair. It is a devastating disease with a rapidly fatal outcome. The aim of our study is to identify cancer stem cells which could specifically be targeted for treatment.
By investigating the Sonic Hedgehog pathway we found a significantly increased Gli-1 expression in MPM tumors compared to normal pleura, indicating stem cell signaling is active in MPM tumors. The stem cell signaling was maintained in primary MPM cell cultures since cyclopamine but not tomatidine could inhibit cell growth and Gli-1 expression.
To identify the stem cell component of tumors we used a functional approach based on the ability of cancer stem cells to efflux Hoechst33342 ("side population" (SP)). Using this functional approach we were able to isolate a SP from ZL55 mesothelioma cells. Sorted ZL55 SP gave rise to a SP and a non-side population (NSP), suggesting that the SP includes cells with self-renewal properties, whereas the ZL55 NSP gave rise only to a NSP. Similar results were obtained for two primary mesothelioma cultures. By characterizing the ZL55 SP and NSP we found an increased expression of ABCG2, a drug transporter responsible for the SP phenotype, and the stem cell maintenance gene Sox2 in the SP compared to NSP. This phenomenon was accompanied by a decreased expression in SP of differentiation markers mesothelin and N-cadherin.
Taken together these results indicate that cells with stem cell renewal properties are present in mesothelioma.

38

The major vault protein and ionizing radiation

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Vaults are large ribonucleoprotein particles, which are highly conserved. Main component of this complex is the major vault protein (MVP). The exact role of vaults is still unsolved but they are involved in the DNA damage stress response since treatment with DNA damaging agents leads to enhanced MVP-expression [1]. Recent clinical studies revealed that high levels of MVP is an adverse prognostic factor for radiotherapy outcome [2]. Additionally, the MVP was proposed to directly interact with the tumor suppressor PTEN during nuclear PTEN import [3]. Here we investigated MVP regulation and resulting consequences as part of the stress response to ionizing radiation (IR). We demonstrated a p53-dependent, dose and time dependent increase of the cellular MVP level in response to irradiation. Experiments performed with MVP-directed siRNA revealed that downregulation of MVP sensitizes to IR, but only in cells with both wildtype p53 and PTEN. Downregulation of MVP did not affect the IR-regulated phosphorylation status of PKB/Akt and ERK. However, downregulation of MVP correlated with decreased levels of Rad51. Strikingly, PTEN, which was proposed to be imported into the nucleus by the MVP, acts as a transactivator of Rad51.
Our study points to a novel link between MVP, p53 and PTEN as part of the DNA damage stress response and IR-induced cell death in cancer. However, the role of MVP in the response to IR in normal tissue still needs further investigations. With those insights MVP could act as both a prognostic factor and a promising target to improve radiotherapy outcome.

1. Shimamoto, Y., et al., Direct activation of the human major vault protein gene by DNA-damaging agents. Oncol Rep, 2006. 15(3): p. 645-52.
2. Silva, P., et al., Tumor expression of major vault protein is an adverse prognostic factor for radiotherapy outcome in oropharyngeal carcinoma. Int J Radiat Oncol Biol Phys, 2007. 69(1): p. 133-40.
3. Chung, J.H., M.E. Ginn-Pease, and C. Eng, Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) has nuclear localization signal-like sequences for nuclear import mediated by major vault protein. Cancer Res, 2005. 65(10): p. 4108-16.

39

Identification of a potential melanoma tumor suppressor gene with differential analysis of transcripts with alternative splicing technology

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Melanoma is an aggressive cancer with a propensity to cause widespread metastatic disease. New methods based on the study of global gene expression are needed in order to understand the events underlying melanoma carcinogenesis.
Alternative splicing events in benign nevus and metastatic melanomas were studied using a new global gene profiling technology: Differential Analysis of Transcripts with Alternative Splicing (DATAS). This genomic analysis confirmed by quantitative PCR assays revealed that the expression of a long isoform of BCSC-1 was consistently downregulated in patients with metastatic melanoma. The analysis of BCSC-1 expression in a cohort of 70 skin biopsies from patients with benign nevus, atypical nevus, primary melanoma or metastatic melanoma revealed that BCSC-1 expression was decreased in melanoma patients in comparison with healthy donors. Comparable results were obtained in melanoma cell lines.
Ectopic expression of the long isoform of BCSC-1 in several human melanoma cell lines decreases their proliferation and induces a block in the G2/M phase of the cell cycle. In contrast the short isoform of BSCS-1 is not downregulated in tumors and does not affect cell proliferation. Invasion assays are ongoing to establish if BCSC-1 regulates only proliferation or affects also invasion potential. Using immunofluorescence analysis, BCSC-1 appears to be predominantly a cytosolic protein.
Experiments in the B16 murine melanoma model demonstrate that the ectopic expression of the long isoform of BCSC-1 decreases metastasis in vivo.
We conclude that the long isoform of BCSC-1 is downregulated in metastatic melanoma and may have a role in the pathogenesis of melanoma.

40

Neonatal experimental infection induces tolerance to Helicobacter pylori

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Helicobacter pylori is a gram-negative bacterium that colonizes the human gastric epithelium in a persistent manner and has been associated with the development of various gastro-intestinal diseases including gastric cancer. However, age of infection may play a critical role in disease outcome. Here we show that experimental infection with a highly immunogenic Helicobacter strain during the neonatal period is able to prevent the inflammatory and adaptive immune responses usually observed in adult C57BL/6 mice and the subsequent development of pre-cancerous lesions, resulting in a non-pathogenic coexistence of bacteria and host. Remarkably, Helicobacter-specific tolerance is not limited to the neonatal period, but is maintained during adulthood.
Continued tolerance to Helicobacter infection requires permanent antigenic stimulation, as antibiotic eradication therapy prior to re-infection four weeks later breaks the tolerance. Because T- regulatory cells (Tregs) control autoimmunity and excessive immune responses to infection in the periphery and have been implicated in the neonatal induction of tolerance to grafts and viruses, we assessed their possible role in the development of tolerance to Helicobacter. Depletion of Foxp3+ T-regs in a transgenic mouse expressing the diphtheria toxin receptor under the control of the Foxp3 promoter indeed breaks the tolerance. The depleted mice are characterized by significantly enhanced inflammatory and adaptive immune responses compared to their non-depleted littermates; they show signs of early preneoplastic lesions and have cleared the infection already 1 month p.i., supporting the notion that newborn T-regulatory mechanisms are essential in the suppression of T cell effector responses to Helicobacter infection.

41

Angiogenesis and lymphangiogenesis are downregulated in primary breast cancer

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Angiogenesis and lymphangiogenesis are considered to play key roles in tumor growth, progression and metastasis. However targeting tumor angiogenesis in clinical trials showed only modest efficacy. We therefore scrutinized the concept of tumor angiogenesis and lymphangiogenesis by analyzing the expression of crucial markers involved in these processes by quantitative Real Time RT-PCR in primary breast cancer. We found decreased mRNA amounts of major angiogenic and lymphangiogenic factors in tumor compared to healthy tissues, whereas antiangiogenic factors were upregulated. Concomitantly, in breast cancer tissues angiogenic and lymphangiogenic receptors were downregulated and the amounts of endothelial and lymphatic endothelial cell were reduced. This antiangiogenic, antilymphangiogenic microenvironment was even more pronounced in grade 3 tumors than in grade 1 tumors. Thus, primary breast tumors are not a site of highly active angiogenesis and lymphangiogenesis. Selection for

tumor cells that survive with minimal vascular supply may account for this observation in clinical apparent tumors.

42

Radiotherapy: Implications for immunotherapy

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Purpose. Radiotherapy is standard in many cancer therapy protocols to date. Recent studies have shown that therapeutic irradiation not only enhances antigen presentation by MHC class I molecule upregulation but also results in the presentation of novel epitopes derived from radiation induced proteins. We propose here that radiation induces the expression of cancer testis antigens in various malignancies, which may result in an immune response. Cancer testis antigens (CTA) are promising targets for cancer immunotherapy.

Experimental Design. To test this hypothesis, we irradiated multiple cancer cell lines, which do not to express CTAs under standard culture conditions, with g-radiation from a 60Co source. The cells were then harvested at different time points after irradiation and the induction of CTAs and the MHC class I expression was analyzed.

Results. Using RT-PCR and immunofluorescence, we found that irradiation induced the expression of CTAs in breast-, lung-, prostate cancer, sarcoma and melanoma. The induction of various CTAs was observed in a randomized fashion and this induction was dose and time dependent. In addition, g-radiation increased the expression of surface MHC class I molecules in a dose and time dependent manner.

Discussion. Our results suggest that a combination of radio- and immunotherapy is a promising and novel approach for the treatment of cancer.

43

Bisphosphonate-liposomes reduce macrophage mediated chemoresistance in cancer cells

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Tumor associated macrophages (TAMs) are known to augment cancer properties e.g., cancer cell migration, invasion, and tumor angiogenesis. Moreover, it has been shown that tumors regress when treated with macrophage depleting agents such as bisphosphonate-liposomes e.g., clodrolip. Considering these diverse effects of TAMs in tumor progression we asked if TAMs also play a role in tumor chemoresistance. In our initial studies we observed decreased apoptosis of cancer cells when they were treated with doxorubicin and factors secreted by macrophages, in contrast to doxorubicin alone. Interestingly, the anti-apoptotic effect of macrophages on cancer cells towards doxorubicin can be reduced by bisphosphonate-liposomes. To seek the molecular mechanism behind this effect we tested the activation of the most prominent receptor tyrosine kinases (RTKs) in cancer cells, EGFR and c-Met, in response to macrophages. We found an increase in EGFR and c-Met activation in response to macrophage -secreted factors which coincides with an increase in Erk and Akt activation. We also observed increased EGF production by macrophages when they were incubated with factors secreted by cancer cells and this effect was attenuated upon bisphosphonate treatment. Therefore, it is attractive to hypothesize that soluble factors secreted by macrophages - presumably growth factors such as EGF and HGF - exert anti-apoptotic effects on cancer cells. Moreover, we can also show that bisphosphonate-liposomes can reduce this anti apoptotic effect. We are currently studying the inhibitory mechanism of bisphosphonate-liposomes at the molecular level. We also look forward to apply bisphosphonate-liposomes in orthotopic murine tumor models as an adjuvant therapy with doxorubicin or other chemotherapeutic agents.

44

Tumor associated macrophages promote tumor invasion by induction of epithelial-mesenchymal transition in F9-teratocarcinoma tumors

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Infiltration of tumors by tumor-associated macrophages (TAMs) is associated with aggressive disease progression. TAMs are known to stimulate pro-tumorigenic processes such as tumor-angiogenesis, tissue remodeling and tumor cell dissemination. Tumor cell dissemination requires motility and invasion which are acquired by epithelial cells that can undergo epithelial-mesenchymal transition (EMT).
EMT is characterized by loss of epithelial- and gain of mesenchymal cell properties. Loss of epithelial properties is partially manifested through loss of epithelial cadherin (E-cadherin). Loss of E-cadherin can result from epigenetic changes or cytokine mediated repression. One consequence is release of the transcriptional co-activator beta-catenin, which can lead to induction of a transcriptional program regulating expression of mesenchymal proteins. As a consequence the cells gain mesenchymal properties and become motile and invasive. TAMs produce a number of cytokines potentially regulating EMT, thus we propose that TAMs can stimulate tumor invasion by inducing EMT in epithelial cancer cells. To investigate this we analyzed F9-teratocarcinoma tumors generated in control mice or mice depleted of macrophages for features characteristic of EMT. Preliminary gene expression- and immunohistochemical analysis revealed an inverse correlation between macrophage infiltration and expression of E-cadherin. Conversely, the analysis manifested a direct correlation between infiltration and expression of mesenchymal markers. We could further show that loss of E-cadherin coincides with the activation of beta-catenin. Data obtained from this study support the hypothesis that TAMs can induce EMT, which, in turn, may induce tumor cell invasion in murine teratocarcinomas. This observation makes TAMs an attractive target for new anti-cancer therapies.

45

GOLPH2 expression in liver tumours and its value as a serum marker in Hepatitis C virus induced hepatocellular carcinomas

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Hepatocellular Carcinomas (HCC) and Bile Duct Carcinomas (BDC) have a poor prognosis. Therefore, surveillance strategies including sensitive and specific serum markers for early detection are needed. Recently, GOLPH2 has been proposed as a serum marker for HCC, but GOLPH2 expression data in liver tissues was not available. Using tissue microarrays and immunohistochemistry we semiquantitatively analysed GOLPH2 protein expression in patients with HCC (n=170), benign liver tumours (n=22) BDC (n=114) and normal liver tissue (n=105). A newly designed sandwich ELISA was used to analyse GOLPH2 levels in the sera of patients with HCC (n=18), HCV (n=10), BDC (n=5) and healthy control persons (n=12). 121/170 (71%) HCC showed strong GOLPH2 expression, which was significantly associated with a higher tumour grade (p=0.01). 97/114 (85%) BDCs showed a strong GOLPH2 expression which proved to be an independent prognostic factor for overall-survival ($p<0.05$). GOLPH2 serum levels were significantly higher in HCC patients than in sera of healthy controls ($p<0.05$). Furthermore, patients with HCV induced HCC, despite having low AFP serum levels, displayed significantly elevated GOLPH2 levels ($p<0.05$). In addition, strong GOLPH2 expression in non-tumourous liver tissue correlated significantly with the presence of HCV infection ($p<0.01$), suggesting GOLPH2 upregulation by viral infection.
Conclusions. GOLPH2 protein is highly expressed in tissues of HCC and BDC. Significant GOLPH2 levels are detectable and quantifiable in the serum by ELISA. In Hepatitis C genotype 1b, serial ELISA measurements in the course of the disease appear to be a promising complementary serum marker in the surveillance of HCC.

46

Selective sensitization of cancer cells to high dose chemotherapy

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Specifically increasing the tolerance of normal cells to cancer chemotherapy agents reduces the side effect, and allows higher dose of agents for therapy, therefore, selectively sensitizes cancer cells to therapy. Our aim is to explore the differences of cell cycle profile between cancer cells and normal cells under different conditions, and use the differences for efficient killing of cancer cells with high dose of chemotherapy agents and selective protection of normal cells at the same time. Short-time (8-15 hours) exposure of high doses (30-80uM) of cisplatin, a cell cycle-specific drug inducing intra- and inter-strand crosslinks of DNA, killed cultured human mesothelioma (ZL55), human lung cancer (A549) and normal human mesothelial cells totally. However, we found that normal mesothelial cells but not cancer cells survived the high dose cisplatin treatment without loss their ability for proliferation when they were pretreated with a short-time (2 to 8 hours) serum starvation (0% serum). Further analysis show that normal mesothelial cells are much more sensitive to the concentrations of serum and glucose in medium than cancer cells: they arrest their cell cycle in a quiescent G0/G1 phase immediately upon serum starvation while cancer cells still underwent DNA synthesis and cell cycle, and were consequently killed by cisplatin. Hence, our results suggest that specifically protecting normal cells but not cancer cells from high dose of cell cycle-specific drugs by selectively arresting normal cells in a quiescent state may be an efficient way to improve the efficacy of therapy.

47

Claudin-1 protein expression is a prognostic marker in renal cell carcinomas

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Aims. We aimed to assess the diagnostic or prognostic significance of the tight junction protein claudin-1 in renal cell carcinoma.
Methods. Protein expression of claudin-1 was immunohistochemically assessed using a TMA format (n=318) and correlated to clinicopathologic tumor parameters including patient survival.
Results. Claudin-1 was found in 29.9% of renal cell cancer cases. Clear cell carcinomas were predominantly negative for claudin-1, whereas papillary tumors were positive. Claudin-1 expression was associated with markers of unfavorable tumor biology in clear cell renal cell carcinoma and it was a prognosticator of shortened disease-specific patient survival (p=0.008) in the subgroup of clear cell renal cell carcinoma, which also remained significant in multivariate analyses in the clinically important subgroups of nonmetastasized or asymptomatic patients.
Conclusions. Claudin-1 expression is an independent prognostic marker of shortened disease-specific patient survival in clinically relevant subgroups of clear cell renal cell carcinoma. Claudin-1 is also expressed in the majority of papillary renal cell carcinomas, which suggests a diagnostic value or a possible use as a therapy target. Functional studies are warranted to clarify the different roles of claudin-1 expression in these histologic subtypes of renal cell carcinoma.

Fritzsche FR, Oelrich B, Johannsen M, Kristiansen I, Moch H, Jung K, Kristiansen G. Claudin-1 protein expression is a prognostic marker in renal cell carcinomas. Clin Cancer Res. 2008 Nov 1;14(21):7035-42

48

Treatment with Cisplatin-Pemetrexed induces senescence pathways in malignant pleural mesothelioma tumor samples

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Background. Malignant pleural mesothelioma (MPM) is an aggressive tumor characterized by chemotherapy resistance. One of the causes could be chemotherapy-induced senescence. The aim of this study was to assess the expression of senescence pathways in MPM tumor samples taken before and after treatment with cisplatin-pemetrexed.
Materials and Methods. RNA was extracted from 20 MPM tumor samples taken from patients from before and after neo-adjuvant treatment (total of 10 patients).Tumor content was assessed by measuring expression level of mesothelioma markers mesothelin, calretinin and podoplanin relative to histone by real time PCR. The expression of fibroblast activation protein (FAP) was also analyzed. Gene expression was validated by Western blot. A tumor marker expression score was determined and compared to H&E stained slides. Senescence pathways were assessed by quantifying the expression of p21, plasminogen activator inhibitor-1 (PAI-1) for the p21-p53 pathway, IGFbPrP1 for the IGF pathway and ALDH3A for the IFN pathway. A p21-PAI1 and a general senescence score were determined.
Results. MPM tumor markers expression in 20 MPM samples demonstrated correlation to tumor to stroma ratio by H&E staining. Three samples with a MPM tumor marker score of less then 10% were excluded from further analysis. FAP expression was detected in all samples. A significant increase in the p21-PAI1 (p=0.047) and general senescence (p=0.021) scores were observed after chemotherapy.
Conclusions. The expression of senescence markers, in particular in the p21-p53 pathway is increased after treatment with cisplatin-pemetrexed.

49

Translesion synthesis of O-6-methylguanine by human DNA polymerase lambda

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O-6-methylguanine (O-6-mG), a DNA lesion produced by alkylating agents, is a strong replicative block for DNA polymerases (pols). It has been shown that all pols tested insert the correct cytosine and incorrect thymine with similar efficiency opposite O-6-mG. Such frequent mismatches can result in G:C→A:T transition mutations upon the next replication round potentially leading to cancer. On the other hand replication of damaged DNA (translesion synthesis, TLS) is achieved by specialized pols (belonging to the Y or X family pols) to overcome such dangerous situations. Pol lambda together with pol beta, belong to the X-family and both enzymes have important roles in base excision repair. In the case of pol lambda it is known the that fidelity of TLS over another important lesion 8-oxo G is enhanced over 100 fold by auxiliary proteins Proliferating cell Nuclear Antigen (PCNA) and Replication protein A (RP-A) (1,2). In addition to the essential roles of RP-A in DNA repli cation, DNA repair and DNA recombination, RP-A was also found to be essential in cell-cycle checkpoint and DNA damage checkpoint signaling via activation of checkpoints. In this work we demonstrate that RP-A has a crucial role in negatively regulating the incorrect translesion synthesis by pol lambda across the O-6mG, thus preventing the incorrect incorporation of a thymidine opposite O-6mG. A possible role of RP-A as a fidelity clamp (3) will be discussed, since it selectively prevented misincorporation of an incorrect nucleotide by pol lambda, without affecting its correct incorporation.

1. Maga, G., Villani, G., Crespan, E., Wimmer, U., Ferrari, E. Bertocci, B. and Hübscher, U.: Nature, 447, 606-609, 2007.
2. Maga, G., Crespan, E., Wimmer, U., van Loon, B., Amoroso, A., Mondello, C., Ferrari, E., Belgiovine, C., Villani, G., Hübscher, U.: Proc Natl Acad Sci U S A. 2008 Dec 30;105(52):20689-94. Epub 2008 Dec 22.
3. Maga, G., Shevelev, I., Villani, G., Spadari, S. and Hübscher, U., Nucl. Acids Res., 34, 1405 – 1415 (2006).

50

The microtubule system as a target for the prevention of tumor cell dissemination

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Dissemination of tumor cells requires a certain level of cell motility and an ability to degrade the extracellular matrix. An intact microtubule system is critical for both cell proliferation and cell invasion and this makes the microtubules an attractive target for anti-cancer therapy. Epothilones are a class of microtubule stabilizing agents (MSAs), which are highly potent in multidrug resistant tumors and less toxic than taxanes. Patupilone (Epothilone B) is one of the compounds from this group with an anti-tumor activity at low nanomolar concentrations and radio-sensitizing properties both in vitro and in vivo. Few evidence exists that MSAs have anti-metastatic effect. The goal of the present study is to assess the effect of microtubule interference on the invasiveness of tumor cells, using the clinical relevant compound patupilone alone and in combination with ionizing radiation (IR).
We assessed cell invasion in vitro after single and combined treatment with patupilone and IR, as well as the treatment response on the level of toxicity and metalloproteinases (MMPs) function. Treatment with high doses of patupilone and IR decreased cell invasion in vitro due to strong inhibition of cell motility despite elevated MMP activities. Treatment with low doses of patupilone (doses that did not have anti-proliferative and cell-cycle altering effect) decreased the expression of MMPs and antagonized an IR-induced increase of MMP activity. These data suggest that the potent treatment combination of IR with patupilone is also a promising treatment modality against metastatic outgrowth.

51

The problem of missense mutations and rare polymorphisms in the multiple endocrine neoplasia type 2 syndrome

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Background. Molecular-genetic screening of cancer-susceptibility genes identifies at risk patients and guides gene/mutation specific management. Medullary thyroid carcinoma (MTC) might occur sporadically or as part of MEN2 syndrome caused by germline mutation in RET gene. Crucial is the distinction between a disease-causing mutation and polymorphic variant. Prophylactic thyroidectomy decreases MTC-related mortality of MEN2 patients and is therefore recommended in carriers of most mutations. But for rare missense variants the problem is the definition, which detected germline variant is disease-causing mutation.

Patients and Methods. The European-American-Pheochromocytoma-Paraganglioma-registry (EAPR) includes phenotype and genotype data (RET, VHL, SDHB/C/D) of pheochromocytoma/paraganglioma patients. The RET p.Tyr791Phe and p.Ser649Leu variants came recently to our attention. First, in a healthy relative of a SDHC p.Arg72Cys mutation carrier affected by tympanicum paragnaglioma the RET p.Tyr791Phe variant was incidentally identified. Second, RET p.Ser649Leu variant was detected in a healthy relative screened for the presence of RET p.Cys634Tyr mutation previously found in his brother affected by bilateral pheochromocytoma and MTC. We re-evaluated the clinical and genotype data of all cases harbouring these variants within the EAPR registry and determined the frequency in 1000 control samples.

Results and Conclusions. The prevalence of the two variants within our patients-based registry was not higher then the frequency in the control population. After clinical re-evaluation none of the subjects had evidence of MTC. These findings indicate that the RET p.Tyr791Phe and p.Ser649Leu are polymorphic variants not predisposing for the MEN2 syndrome.

Rare missense variants should be critically reviewed prior application of any specific cancer-susceptibility-gene management/treatment.

52

Expansion of umbilical cord blood mesenchymal stem cells

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Umbilical cord blood (UCB) is known to harbor 2 major types of stem cells, the hematopoietic stem cells and the nonhematopoietic or mesenchymal stem cells (MSC). Under appropriate conditions, MSC can give rise to cells of bone, fat, hepatic lineages etc. Based on this potential, MSC hold promise for clinical applications for regenerative medicine.

MSC were first generated from UCB cryosaved mononuclear cells (MNC) cultured in the presence of early growth factors in stroma –free liquid culture. MNC derived adherent MSC were then enriched in MesenCult medium. Following repeated passages, MSC count increased 357- and 600-folds and CFU-Fibroblasts colonies (CFU-F) increased too (61-513 and 648-697) after 10 and 20 passages respectively. We used the CFU-F assay to demonstrate MSC activity in stromal cell formation in vitro. Phenotypic analysis showed that MSC were negative for hematopoietic antigens (CD45, CD34 and CD14), MHC class-I negative but >95% + for (CD73, CD105, CD29 and CD44).

To demonstrate MSC differentiation capacity in vitro, cells at passage 5 were cultivated in media suitable for growth and differentiation into fat (adipocytes); bone (osteoblasts) and liver (hepatocytes) cells. Following induction, positive staining with oil red O for cells of adipocyte and with alkaline phosphatase for cells of osteoblsts lineages were observed under appropriate conditions. To observe hepatocytes differentiation, the appearance of cells with hexagonal hepatocytic shape was used as primary screening criterion. Hepatocytes like cells expressed albumin, CK 18 and CK14 as assessed by flow cytometry, and RT-PCR. MSC described herein exhibit in vitro properties of multipotent stem cells. The research in this area has a high potential, with respect to future application in patients with end stage liver disease.

Stieger B, Peters RE, Sidler MA,Meier PJ (2006). Hepatocyte transplantation: potential of hepatocyte progenitor cells and bone marrow derived stem cells. Swiss Med Wkly 136: 552-556.

53

Destination and extent of osteosarcoma metastasis is determined by the localization of the primary tumor

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In the contemporary understanding of metastasis, the microenvironment not only of the target organ but also of the primary tumor (PT) is thought to be crucial for determination and preparation of the metastatic niche (1,2). To investigate the impact of the PT site on metastasis in osteosarcoma (OS), the metastatic pattern of orthotopically/intratibially (i.t.), heterotopically/subcutaneously (s.c.) and intravenously (i.v.) injected low (Dunn-lacZ) and highly (LM8-lacZ) metastatic murine OS cells was compared. For an improved detectability of the metastases the OS cells were tagged with the lacZ-gene before inoculation.
Spontaneous LM8-metastasis in the s.c. model was mainly focused to the lung, while liver and ovaries represented the predominant target organs of metastasis in the i.t. and i.v. models. S.c. inoculation of Dunn cells exclusively led to spontaneous micrometastasis (<0.1mm) in lung and liver. In contrast, i.t. and i.v. injected Dunn cells additionally generated macrometastases (>0.1mm) in lung, liver and ovaries. Life-threatening morbidity in the s.c. model seemed to be mainly caused by massive PT growth, whereas in the i.t. and i.v. model metastatic load of livers appeared to be the crucial determinant. In the i.v. model, also spine metastases contributed to life-threatening morbidity due to paralysis of lower extremities.
This study demonstrates for the first time a potential impact of the tumor cell injection site on PT growth capacity, extent and localisation of metastasis and cause of death in OS. This appreciation may be important for the future rational utilization of OS animal models in cancer research.

1. Gupta GP and Massague J; Cell 2006;127:679-95.
2. Kaplan RN; Cancer Res 2006;66:11089-93.

54

Induction of autophagy-dependent cell death is required to restore steroid sensitivity in steroid-resistant ALL

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Glucocorticoid (GC) -resistance is a common feature in patients with acute lymphoblastic leukemia at very high risk for relapse based on their molecular response to chemotherapy. Modulating cell death regulators represents an attractive strategy to subvert drug resistance. We report that subcytotoxic concentrations of the BH3-mimetic obatoclax resensitize glucocorticoid (GC)-resistant ALL to dexamethasone. This effect did not require mitochondrial membrane depolarization, was maintained in Bax/Bak- or caspase-9 deficient ALL cells, and resulted in rapid induction of autophagy. Resistance to dexamethasone and obatoclax was restored either by pharmacological inhibition of autophagy using 3-MA or bafilomycin as well as by downregulation of the essential autophagy genes Beclin-1 or ATG-7 using siRNA. The cytotoxic effect of the combination of obatoclax and dexamethasone was markedly impaired in clonogenic assays after exposure to 3-MA or downregulation of Beclin-1. Obatoclax also induced autophagy dependent cell death in Bax/Bak deficient mouse embryonal fibroblasts. Similarly, we show that GC-resensitization by rapamycin was dependent on autophagy, underscoring the importance of autophagy induction in resensitization to GCs. In a xenograft model of refractory ALL, the combination of obatoclax and dexamethasone halted leukemic progression, These data provide a compelling rationale for clinical translation of this combinatorial strategy in drug-resistant ALL. Our results also call for extensive preclinical studies when considering approaches to target the autophagy pathway in order to better understand the cellular context in which autophagy will contribute to effector cell death mechanisms in cancer.

55

GOLM1 protein expression as a novel tissue biomarker for prostate cancer

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Aims. Prostate carcinoma is the second most fatal cancer in males in western countries. Currently the diagnosis of this disease is based on the histological confirmation of cancer infiltrates in prostate biopsies. The major challenge is therefore the identification of a reliable marker protein to increase diagnostic security.
Methods. Using array based transcript analysis of matched prostate cancer tissue samples from 42 patients that underwent radical prostatectomy we have previously identified golgi membrane protein 1 (GOLM1) mRNA among others as being overexpressed in tumor tissue.
In the current study we analyzed the protein expression level of this potential marker in a larger cohort of prostate cancer patients on a tissue microarray comprising 614 tissue specimens and compared the expression to AMACR.
Results. GOLM1 is expressed in normal as well as in tumor tissue but the median intensity of the staining was considerably higher in 92.4% of the tumors. Moreover, GOLM1 was upregulated in 84% of the AMACR-negative cases. As the function of GOLM1 is largely unknown we further assessed its tumorbiological significance in prostate cancer cell lines concerning its ability to influence cell proliferation and invasion using a siRNA based approach. Preliminary data indicate that GOLM1 knockdown may block the proliferation of the DU-145 cell line.
Conclusions. These data suggest GOLM1 as a novel tissue biomarker for prostate cancer that allows the detection of 99.2% of all prostate cancer cases in combination with the already known positive marker for malignancy alpha-methylacyl-CoA racemase (AMACR).

56

PTEN involvment in Eph B1 receptors signal termination

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Eph receptors and their ligands (ephrins) play an important role in axonal guidance, embryonic patterning and angiogenesis. In the last decade, they have also been shown to be key players in carcinogenesis and tumor neovascularization. To date only few studies have focused on the mechanisms of Eph receptor signal termination. In a previous work, our group has shown that juxtacrine stimulation with ephrinB2/Fc leads to EphB1 poly-ubiquitination by the E3 ubiquitin ligase Cbl and its subsequent degradation through the lysosomal pathway. In the present study we demonstrated that in CHO-EphB1 cells, the tumor suppressor PTEN (Phosphatase and Tensin Homolog) is phosphorylated and constitutively linked to Cbl. Upon EphB1 stimulation, phospho-PTEN is dephosphorylated allowing the disruption of the Cbl/PTEN complex. Confocal microscopy experiments demonstrated that in stimulated cells, Cbl is recruited to EphB1 receptors whereas dephosphorylated PTEN is translocated at the edge of th e cell. Overexpression of G129R PTEN mutant (lipid and protein phosphatase dead) resulted in a defect of Cbl/PTEN complex disruption, Cbl activation and EphB1 degradation. These effects were not seen in cells transfected with the lipid phosphatase dead G129E mutant. To our knowledge, this is the first study showing that the tumor suppressor PTEN plays a critical role in signal termination of an Eph receptor, and that its protein phosphatase activity is crucial for this process.

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Science 1998 Jun 5;280(5369):1614-7
EMBO J. 1999 Apr 15;18(8):2165-73

57

FASN is overexpressed in the majority of prostate cancer cases and might be an ancillary positive marker of malignancy

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Aims. In previous studies we have identified fatty acid synthase (FASN) overexpression in prostate cancer on mRNA level. The clinical significance and the possible diagnostic value of FASN protein expression are still unknown.
Methods. FASN protein expression was analysed immunohistochemically in 630 clinically characterized prostate cancer cases and compared to adjacent normal prostate tissue. In parallel, the expression of AMACR/p63 and Ki-67 was determined.
Results. FASN was expressed in 98% of prostate cancer cases and in 68.4% it was clearly upregulated in comparison to normal tissue. FASN expression was correlated to higher Ki-67 and AMACR levels, but not to Gleason score, pre-operative PSA levels, pT stage or margin status. Importantly, FASN was upregulated in 60% of AMACR negative cases. No prognostic value of FASN was found.
Conclusions. In confirmation of mRNA data, we found FASN protein overexpressed in prostate cancer. In comparison to AMACR, which is widely diagnostically used as a prostate cancer marker, FASN was inferior due to lower expression rates. However, it might be of use in critical cases as an adjunct positive marker.

Baron A, Migita T, Tang D, Loda M. Fatty acid synthase: a metabolic oncogene in prostate cancer? J Cell Biochem. 2004;91:47-53

Rossi S, Graner E, Febbo P, Weinstein L, Bhattacharya N, Onody T, Bubley G, Balk S, Loda M. Fatty acid synthase expression defines distinct molecular signatures in prostate cancer.
Mol Cancer Res. 2003;1:707-15.

58

Impaired pro-inflammatory cytokine profiling in prostate cancer patients upon induction using peptides within polyomavirus BK-p53 binding regions

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Polyomavirus BK (BKV) oncogenisis is due to the ability of the viral large tumor antigen (LTag) to regulate critical pathways, such as p53, of human cell cycle when infecting non permissive cells. In prostate, cytoplasmic co-localization of BKV LTag and p53 is detectable at proliferative inflammatory atrophy (PIA) stages. It prompted us testing whether an immune response against LTag-p53 binding regions might define a role for BKV immunosurveillance in prostate cancer (PCa). 82 male patients (39 benign prostatic hyperplasia (BPH) and 43 PCa) and 10 healthy gender-matched donors were enrolled. 35/39 BPH (89.7%) and 40/43 PCa (93%) patients clustered in the lower IgG titre range for LTag serology. Moreover, 45.5% (n=15/33) of BPH and 57.2% (n=12/21) of PCa surgically excised specimens were positive for BKV-LTag DNA detection. Stratifying our cohorts of patients based on BKV-LTag DNA detection, all BPH (7/7, 100%) and almost all PCa (9/10, 90%) BKV-LTag DNA+ patients clustered w ithin the lowest IgG titre range for LTag serology. PBMCs from HLA-A*0201 patients were ex vivo stimulated with two HLA-A*0201 restricted peptides within p53-LTag binding domains (LTag406 and LTag579). Evidence of a proinflammatory and lytic activity impairment in favor to an immunoregulatory function was better seen in PCa than BPH patients by plotting cytokine gene expression (IL-10/IFN-g) against LTag IgG serology upon both peptides LTag406 (r=0.71, p=0.002) and LTag579 (r=0.61, p=0.001) induction. Among PCa, 41.7% of BKV-LTag DNA+ patients upon LTag406 and 28.5% upon LTag579 peptides induction showed evidence of immunoregulatory activity and higher Gleason score.

CD45RA+/CCR7+/- T lymphocyte responses against p53-binding domains of the human polyomavirus BK large tumor antigen in HLA-A*0201+ BKV-seropositive donors. J Transl Med 2006, 4:47.

59

Optimized clustering methods for analysis of heterogeneous oncogenomic data collections

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Profiling of genomic imbalances in malignant neoplasias offers insights into disease classification and risk stratification for cancer patients, and has identified cancer related genes. The most successful oncogenomic screening techniques have been comparative genomic hybridization (CGH) variants.
While most CGH projects include a limited number of cases, the analysis of thousands of heterogeneous malignancies promises general insights in oncogenetic pathways. With the Progenetix database, we have established the largest public collection of CGH profiles. In one of our current approaches, we use this data for optimization of clustering tools for genomic data segmentation and visualization. Clustering methods use various distance measurements to compute similarity between data vectors. Although distance methods (e.g. Euclidean, Hamming distance) have been used on CGH data, they do not consider fully the problems of A) binary data, with B) dependency between neighboring events. Previously, clustering of chromosomal CGH data with a marker-based approach had been proposed, which however required sub-setting large data sets using prior histopathological knowledge.
Here, we are developing a method to cluster heterogeneous CGH data. Markers are defined as continuous type of aberration for gains and loss separately, allowing for independent gain/loss markers to occupy the same genomic space. In limited testing, the method has successfully aligned patient profiles independent if derived from heterogeneous or homogeneous data sets. This approach may be applied to data e.g. from clinically different carcinomas, to identify tumors with common genomic pathways and therefore possibly related molecular targets, suitable for therapeutic intervention.

Baudis M: Genomic imbalances in 5918 malignant epithelial tumors: an explorative meta-analysis of chromosomal CGH data. BMC Cancer 2007, 7:226.

Liu J, Mohammed J, Carter J, Ranka S, Kahveci T, Baudis M: Distance-based clustering of CGH data. Bioinformatics 2006, 22:1971-1978.

Baudis M, Cleary ML: Progenetix.net: an online repository for molecular cytogenetic aberration data. Bioinformatics 2001, 12:1228-1229.

60

Targeting different levels of PAX3/FKHR oncogenicity by combination treatment

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Alveolar rhabdomyosarcoma (aRMS) is a soft tissue malignancy characterized by specific translocations generating the chimeric transcription factor PAX3/FKHR. As PAX3/FKHR is a stronger transactivator compared to wild-type PAX3 its oncogenic properties are hypothesized to result from transcriptional deregulation. In addition, recent studies have shown that PAX3/FKHR influences gene expression through selective proteasomal-degradation of the EGR1 transcription factor (Roeb, Boyer et al. 2007). Therefore, we set out to identify targets of EGR1 whose expression is controlled by PAX3/FKHR.
Here we report that the EGR1 target gene p21Cip1 is repressed via PAX3/FKHR-mediated destabilization of EGR1 and provide evidence that downregulation of p21 contributes to survival of aRMS cells. As the transcriptional activity of PAX3/FKHR can be targeted by the kinase inhibitor PKC412 (Amstutz, Wachtel et al. 2008), we reasoned that a combination treatment with small molecule inhibitors that reactivate p21 expression could augment the antitumorigenic effect of the treatment.Among a panel of histone deacetylase inhibitors known to mediate upregulation of p21, we could identify valproic acid (VPA) as a promising combination agent for PKC412. Treatment of aRMS cells with PKC412 and VPA showed synergistic induction of apoptotic cell death in vitro and in vivo which resulted in suppressed tumor growth in an aRMS xenograft model. In addition, we could demonstrate that combination treatment with VPA reactivated p21 expression levels in aRMS cells and xenograft tumors. Therefore, our results suggest that the combination of PKC412 with VPA could be a new and effective approach for aRMS treatment as the two inhibitors complement each other by targeting different levels of PAX3/FKHR oncogene activity.

Amstutz, R., M. Wachtel, et al. (2008). Phosphorylation regulates transcriptional activity of PAX3/FKHR and reveals novel therapeutic possibilities.
Cancer Res 68(10): 3767-76.

Roeb, W., A. Boyer, et al. (2007). PAX3-FOXO1 controls expression of the p57Kip2 cell-cycle regulator through degradation of EGR1.
Proc Natl Acad Sci U S A 104(46): 18085-90.

61

The metalloprotease CD10 is a VHL regulated, drugable HIF-target that is abundant in the serum of clear cell renal cell carcinoma patients

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Loss of von Hippel-Lindau (VHL) tumor suppressor function is a frequent event in the development of inherited and sporadic clear cell Renal Cell Carcinoma (ccRCC) and is directly linked to the activation of Hypoxia Inducible Factor (HIF). HIF target genes, such as CAIX or VEGF, represent potential diagnostic and therapeutic markers in human tumors. Like CAIX, the metalloprotease CD10 is frequently expressed in VHL deficient ccRCC, however, specific insights into the regulation of CD10 expression are missing. Here we show that both loss of pVHL function and hypoxia induce CD10 promoter activity and protein expression in a HIF-dependent manner. Knockdown of HIF2alpha reduced CD10 expression after loss of pVHL. Cell invasion assays revealed a diminished invasive behavior of pVHL negative 786-O cells after treatment with the CD10 specific inhibitor Thiorphan. Immunohistochemical and VHL sequence analyses on human ccRCC unveiled significant correlations between CD10 expression, HIF stabilization and loss-of-function mutations of VHL. CD10 serum levels were significantly elevated in sera from ccRCC patients compared to healthy controls. Our results establish CD10 as a novel HIF-target gene whose activation is directly dependent on functional pVHL. We conclude that in ccRCC, CD10 is driven by HIF2alpha following VHL loss. Its influence on tumor cell invasion and elevated protein levels in patients' sera suggest CD10 as a potential diagnostic marker and novel therapeutic target in ccRCC.

62

Analysis of signal transduction networks in the insulin signaling pathway in drosophila melanogaster cells using systematic RNA interference screens

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The insulin signaling pathway controls key aspects of cellular growth, survival and proliferation. Its operation is tightly coupled to and coordinated with nutrient and oxygen sensing pathways. Mutations of several pathway components are also implicated in various cancers. Despite the importance of these pathways for normal cell and organismal function we know very little about how hormonal signaling is interconnected with nutrient and oxygen sensing cues. A better understanding of how these different signaling inputs are coordinated into a network will also expand the therapeutic potential of treatments of diseases that are a result of mutational inactivation of one or more components of this network. The development of large scale RNAi approaches to identify potential anti-cancer targets is gaining increasing importance with the possibility of novel combinatorial chemical and genetic interactions being unearthed.
With Drosophila as a model organism, we attempted to identify novel interactors of the well-known tumor suppressor PTEN, using a cell-based RNAi microarray approach coupled to the principle of synthetic lethality. Screening of the Drosophila kinase/phosphatase protein families in Kc167 cells for synthetic lethal interactors of PTEN led to the discovery of proteins involved in key cellular processes including cell division, growth, and energy homeostasis pathways, as well as some proteins of unknown function. Validation of the targets using secondary screens and functional assays is ongoing.

63

Leukemia-initiating cells are frequent in very high risk childhood precursor B acute lymphoblastic leukemia

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Poor risk acute lymphoblastic leukemia (ALL) has been proposed to arise from a limited immature leukemia initiating cell (LIC) compartment that may convey treatment resistance. Functional assessment of LIC frequency using syngeneic mouse leukemia models and recent data showing that different ALL subpopulations, sorted based on expression of CD19, CD34 and CD38, can reconstitute ALL in NOD/SCID mice challenge this view (1,2). Here we show that ALL cells from very high risk (VHR) and from standard risk (SR) patients display heterogeneous immunophenotypes, some having a large CD34+/CD19+ compartment while others have a large CD34-/CD19+ compartment.
Aldehyde dehydrogenase activity (ALDH), which is a marker of hematopoietic stem cell and progenitor function, was also heterogeneous. Thus it appears unlikely that the LIC compartment can be identified in ALL with such markers. Using xenotransplantation of primary ALL cells by intrafemoral injection in immunodeficient NOD/scid-IL2gamma null (NOG) mice, we evaluated the number of unsorted cells required for reconstitution of VHR-ALL. One million of unsorted ALL cells generated leukemia in NOG mice in 5/5 cases, and 100 cells were sufficient for engraftment (in 2/5 cases) without conditioning, despite the xenograft barrier. The leukemia immunophenotype as well as the hepatosplenic and bone marrow involvement pattern was conserved, and secondary transplantations demonstrate conserved self renewal properties. Experiments with injection of 10 or less cells are ongoing. Based on our observations and recent reports it is conceivable that most if not all ALL cells retain stem cell properties to give rise to the leukemia.

(1) Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A: Tumor growth need not be driven by rare cancer stem cells. Science 2007, 317(5836):337.
(2) le Viseur C, Hotfilder M, Bomken S, Wilson K, Rottgers S, Schrauder A, Rosemann A, Irving J, Stam RW, Shultz LD et al: In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. Cancer cell 2008, 14(1):47-58

64

Assessing the link between mismatch repair and excision repair

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DNA damage can threaten the survival of an organism. One kind of DNA damage are replication errors that are corrected by the mismatch repair (MMR) machinery.
One recently identified interaction partner of MMR proteins is ICE114, which seems to link the mismatch repairosome to other DNA repair pathways. ICE114 was identified as an interaction partner of MutL(alpha), a heterodimer of MLH1 and PMS2 that is believed to couple the mismatch recognition step, mediated by MutS(alpha) or MutS(beta) (heterodimers of MSH2 and MSH6, or MSH2 and MSH3, respectively), to downstream processes that include the removal of the mismatch from the nascent DNA strand, resynthesis of the degraded region and ligation of the remaining nick.
ICE114 encodes an as-yet uncharacterized protein. Bioinformatic analyses predicted that ICE114 might contain a zinc finger motif at its N-terminus and an endonuclease domain of the PD-(D/E)XK superfamily at its C-terminus. Members of this superfamily are involved in DNA repair, recombination and replication. This fact, coupled with the discovery of the ICE114/MutL(alpha) interaction suggests that ICE114 might be involved in DNA metabolism.
The aim is to assess the role of ICE 114 in MMR and DNA excision repair, respectively. What is the exact task of ICE114 in DNA repair? Does it function as an endonuclease as proposed in Kosinski (2005)? Does ICE114 recruit MMR proteins to other DNA repair pathways?

65

Targeting the human papillomavirus E6 oncoprotein by a peptide inhibitor

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High-risk types of human papillomaviruses (HPVs) cause certain human malignancies, in particular cervical cancer. Tumor growth is mediated by the joint function of the viral oncoproteins E6 and E7. We previously found that E6 exerts anti-apoptotic activities which are crucial for the survival of HPV-positive cancer cells. Thus, targeted inhibition of E6 should represent a rational therapeutic strategy to eliminate HPV-positive preneoplastic and neoplastic cells. By screening a randomized peptide expression library, we identified a 15mer peptide, pep11, which selectively binds to the HPV16 E6 protein with high affinity. Mutational analyses revealed that pep11 harbors a novel E6 binding motif which is not found in known cellular E6 binding partners. Importantly, intracellular expression of pep11 efficiently induced apoptosis, selectively in HPV16-positive cancer cell lines. Biochemically, pep11 interfered with E6-mediated degradation of p53. Consequently, pep11 expression restored intracellular p53 levels and led to the induction of p53 downstream target genes. These data indicate that targeted inhibition of E6 activity in HPV-positive cancer cells can re-induce the dormant p53 tumor suppressor pathway, ultimately leading to the apoptotic elimination of HPV-positive cancer cells. We envision that the therapeutic potential of pep11 can be advanced to an application perspective, e.g. by developing pep11-derived proteinaceous drugs for topical application. Furthermore, the pep11/E6 interaction provides a unique basis to identify non-peptide small molecule inhibitors of E6 with pro-apoptotic potential. Such specific E6 inhibitors should act with high therapeutic selectivity, since they target a viral factor which is not present in undiseased cells.

1. Dymalla S et al., A novel peptide motif binding to and blocking the intracellular activity of the human papillomavirus E6 oncoprotein. J Mol Med. in press.
2. Butz K et al., siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus-positive cancer cells. Oncogene. 2003 Sep 4;22(38):5938-45.

66

Novel tumor targeting peptide for rhabdomyosarcoma identifies the surface expressed pro-protein convertase furin as potential therapeutic target

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Current therapies for rhabdomyosarcoma (RMS), the most common soft tissue sarcoma in children, are limited by their systemic toxicity causing serious long lasting side effects. By panning with a phage-displayed cyclic random peptide library on RMS cells in vitro and in vivo, we identified a peptide (RMS-P3) which showed strong binding affinity to RMS cell lines, as well as to several other cultured tumor cell lines. RMS-P3 showed specific binding to RMS xenografts in vivo, and RMS-P3 peptide-mediated targeting of doxorubicin to RMS increased the therapeutic impact by delaying tumor growth at least 2-fold compared to non-targeted doxorubicin treatment alone. The minimal binding motif of RMS-P3 was identified by alanine-scan, and lead to the identification of the pro-protein convertase furin as one target receptor. Based on these results, we are investigating the molecular basis for the targeting specificity of the peptide by analysing the differences in furin expression betwee n normal and tumor tissues and by characterising the physical interaction between RMS-P3 and furin. In conclusion, the identification of a novel and specific tumor targeting peptide, and of its cellular binding protein, will lead to an improved specific treatment for RMS.

67

CD8+ T cell response against a mucin epitope in patients with breast CD8+ cancer

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Mucin 1, encoded by the MUC1 gene, is a tumor-associated antigen expressed on the surface of breast cancer cells. It would be of interest to see whether there is a naturally existing T cell immune response against mucin epitopes in cancer patients.
Using tetramer and interferon-gamma assays, the immune response to one MUC1 peptide epitope in the peripheral blood of breast cancer patients was quantified. The data were compared with the clinical course of the patients. CD8(+) T cells capable of recognizing the HLA-A*0201-restricted STAPPV-HNV epitope were detected in 9 of 19 patients with a frequency ranging 0.01-0.082%. No significant difference was found between the occurrence of epitope-specific CD8(+) T cells of patients with progressive disease and disease-free patients. However, all patients with stable disease showed a specific immune response, including both patients with the highest frequency. The results of this study provide further evidence that a natural specific cellular immune response against this mucin epitope exists in breast cancer patients

1.Kokowski K, Harnack U, Dorn DC, Pecher G. Quantification of the CD8+ T cell response against a mucin epitope in patients with breast cancer. Arch Immunol Ther Exp (Warsz). 2008 Mar-Apr;56(2):141-5. Epub 2008 Mar 31

68

Expression of cancer stem cell markers in malignant pleural mesotheliomas correlates with the histological subtype

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Background. Malignant mesothelioma (MM) is a highly aggressive tumor and the most common malignancy of the pleura. The identification and characterization of cancer stem cell markers, a small population of tumor-initiating cells is one of the major concern in present research. The D2-40 antibody recognizes the 40 kDa sialoglycoprotein podoplanin, which is expressed among others in MM, germ cell neoplasia and fetal testicular gonocytes. The intermediated filament nestin, a gene also expressed in neural progenitor cells and the Polycomb group gene bmi-1 are further tumor-initiated proteins. Also the expression of the transcriptionfactor sox10 and the cytoplasmatic adherens junction protein beta-catenin are under investigation in this study.
Design. Tumour of 352 patients with malignant mesothelioma was used for construction of a tissue microarray with quadruplicate cores (total core number n=1408). Biphasic mesotheliomas were represented by two epithelioid and two sarcomatoid cores. The protein expression of calretinin and of the cancer stem cell markers podoplanin, sox10, nestin, bmi-1 and beta-catenin were analysed by immunohistochemistry, using respective antibodies and a semi-quantitative sum scoring system. Statistical analysis was performed with data dichotomized at the median and chi-squared tests.
Results. Of the 352 malignant mesotheliomas, 117 were of epithelioid, 45 of sarcomatoid and 190 of biphasic subtype. Expression of calretinin was found in 86.9%, podoplanin in 51.3%, sox10 in 58.3%, nestin in 62.7%, bmi-1 in 85.1% and beta-catenin in 58.6% of the tumor. In comparison to previous study calretinin was associated with bmi-1 and podoplanin and with the epitheloid histological subtype (p-value <0.05). Furthermore the expression of sox10 correlates with the expression of beta-catenin and shown a prevalence to the sarcomatoid histological subtype (p-value <0.05).
Conclusion. Cancer stem cell markers are differentially expressed in the histologic subtypes of malignant mesothelioma. In particular, the expression of the transcription factor sox10 and the cytoplasmatic junction protein beta-catenin are associated with the sarcomatoid subtype of MM.

69

Glucagon-like-peptide-1 (GLP-1) receptor as a new diagnostic and therapeutic target in human insulinoma

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Introduction. Neuroendocrine tumors are well vascularized and express specific cell surface markers. The glucagon-like-peptide-receptor-1 (GLP-1R) is selectively overexpressed in human insulinoma. We developed [Lys40(Ahx-DTPA-111In)NH2]-Exendin-4 (In-Exendin), a radiopeptide, which specifically binds to the GLP-1R (1). Using the Rip1Tag2 mouse model of human insulinoma, we investigated the diagnostic and therapeutic potential of In-Exendin alone or in combination with an anti-angiogenic treatment.
A clinical pilot study investigating the use of In-Exendin in the diagnosis of human insulinoma is running (2).
Methods. In the Rip1Tag2 model, In-Exendin was injected as a single agent or co-administered with PTK787, a VEGFR-2 inhibitor. For therapy, mice were injected with 1.1 to 28 Megabecquerels (MBq) In-Exendin as a monotherapy or treated simultaneously with PTK787.
Study patients (n=4) were injected with In-Exendin to visualize insulinomas through SPECT technology.
Results. Using In-Exendin and SPECT-MRI, insulinomas of 2mm could be visualized in the Rip1Tag2 mouse. Treatment of mice with In-Exendin induced a dose-dependent regression with a 95% reduction of tumor volume. However, with 28MBq, kidney toxicity became a relevant concern. Treatment of mice with 1.1MBq In-Exendin together with PTK787 led to a volume reduction of 97% without organ damage.
In patients, In-Exendin was capable to detect insulinomas in vivo.
Conclusion. In this study, we confirmed the role of the GLP-1R as a target for diagnosis and treatment of insulinoma. Insulinomas could be visualized both in the mouse model and in patients. Therapeutically, In-Exendin was successfully used alone or in combination with an anti-angiogenic compound to target insulinomas in the Rip1Tag2 model.

(1) Wicki A, Wild D, Storch D, Seemayer C, Gotthardt M, Behe M, Kneifel S, Mihatsch MJ, Reubi JC, Mäcke HR, Christofori G.
[Lys40(Ahx-DTPA-111In)NH2]-Exendin-4 is a highly efficient radiotherapeutic for glucagon-like peptide-1 receptor-targeted therapy for insulinoma.
Clin Cancer Res. 2007 Jun 15;13(12):3696-705.

(2) Wild D, Mäcke H, Christ E, Gloor B, Reubi JC.
Glucagon-like peptide 1-receptor scans to localize occult insulinomas
N Engl J Med. 2008 Aug 14;359(7):766-8.

70

FBP (FUSE binding protein) as a tumorigenic factor and potential therapeutic target in hepatocellular carcinoma (HCC)

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Recognizing dynamic changes in DNA topology, the single-stranded DNA binding factor FBP (FUSE-binding protein) and its partner FIR (FBP interacting repressor) both function as transcription factors. Previously, we have shown that they antagonistically modulate the expression of the c-myc proto-oncogene. These data suggest a potential tumorigenic function for FBP and FIR. So far, nothing is known about the relevance in human carcinogenesis. We found that both, FBP and FIR are over-expressed in human HCCs, and their expression profiles significantly correlated among each other. However, expression did not correlate with elevated c-myc transcript or protein levels. Tissue-micro arrays (n=2(1)5) revealed a highly significant correlation between the nuclear expression of FBP and the cytoplasmic enrichment of FIR with tumor progression. Furthermore, the expression of both, FBP and FIR significantly correlated among each other, but not with the expression of c-myc in HCCs. Using RNAi nterference the transient inhibition of FBP and FIR in different HCC cell lines did not influence the c-myc expression, but significantly reduced tumor cell viability and proliferation. Interestingly, expression of FBP inversely correlated with survival of HCC patients. Taken together, FBP and FIR are over-expressed and co-regulated during human hepatocarcinogensis. Since both transcription factors do not influence the expression of c-myc, they probably exert their oncogenic properties through the activation of still unknown pro-tumorigenic factors. Since both transcription factors reveal tumorigenic properties in liver cells, they are potential targets for HCC therapy.

71

Inhibition of translesion synthesis reduces the development of chemotherapy resistance

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Background. Malignant pleural mesothelioma (MPM) is most commonly treated with a multimodality therapy including treatment with cisplatin or cisplatin-analogues. Cisplatin adducts can be repaired or, if not repaired, induce replication fork stalling which can by overcome by specific translesion polymerases. Translesion polymerase zeta consists of two subunits, Rev3 is the catalytic- and Rev7 the structural subunit. The translesion polymerase zeta is responsible for the translesion synthesis (TLS) of cisplatin based adducts and the repair of DNA interstrand crosslinks. Rev3 inhibition by antisense treatment confers higher cisplatin sensitivity and lower mutagenicity in immortal human fibroblasts.

Working hypothesis. Down-regulation of Rev 3 sensitizes MPM cells to cisplatin treatment and reduces the formation of cisplatin resistance.

Results. We showed that the expression of Rev3 in human MPM cells is dependent on cell culture confluency and is also affected by cisplatin treatment in a time-dependent manner. Functional inhibition of REV3 by siRNA increased replication fork breakdown as indicated by enhanced H2AX phosphorylation. We generated stable HEK293 and human lung fibroblast (Wi38-SV40) cell lines with decreased REV3 expression. Functional inhibition of REV3 in vitro resulted in increased genotoxic stress as indicated by increased p53 expression, a slower growth rate and increased cisplatin sensitivity. In addition, REV3 inhibition significantly reduced the occurrence of cisplatin resistance in human lung fibroblasts.

Conclusions. We showed that functional inhibition of translesion polymerase zeta by shRNA against REV3 increased replicative stress in human cell lines, resulting in increased cisplatin sensitivity and reduced cisplatin resistance formation.

72

T cell responses in helicobacter-induced gastric preneoplastic pathology

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The outcome of chronic infection with Helicobacter pylori is very different across infected individuals. Whereas the majority remains asymptomatic despite persistent colonization, roughly % 20 develop gastric disorders ranging from chronic gastritis and duodenal ulcers and to gastric adenocarcinoma and MALT lymphoma. Our laboratory is interested in the mechanisms underlying these differences. We utilize the C57Bl6 model, in which we observe the formation of Helicobacter-induced pre-neoplastic changes. In humans, Helicobacter pylori-induced activation of epithelial cells, polymorphonuclear and mononuclear cells leads to a T-helper type (1) (Th(1)) response. The hallmark of a Th(1) response is the production of interferon-gamma (IFN-gamma). Our initial observation suggested that a strong IFN-gamma response is correlated with clearance of the bacterial colonization, but also with induction of preneoplastic gastric lesions. We confirmed this finding in IFN-gamma-/-, TCR-/- (no functional T cells), and RAG-(1)-/- (no mature B and T cells) mice infected by Helicobacter for (1) and 3 months. Also, in RAG-/- background, CD4+ effector T-cells from infected donors induce preneoplastic pathology and clear the infection and this CD4+ effector cell-mediated clearance and pathology depend on IFN-gamma. Overall, our data suggests that the magnitude of the IFN-gamma response was a good indicator of bacterial colonization levels on the one hand, but also susceptibility to Helicobacter –induced preneoplastic epithelial changes on the other. Indeed,IFN-gamma (secreted by produced by CD4+ effector cells) is crucial for clearance of Helicobacter in spontaneous clearance model. It is also necessary to limit bacterial replication under experimental infection conditions.

73

Acquired histone deacetylase (HDAC) inhibitor resistance in colon tumor cells treated with Vorinostat in vitro

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Histone deacetylase inhibitors (HDACi) such as Vorinostat (SAHA; suberoylanilide hydroxamic acid) act epigenetically by regulating gene expression through chromatin remodeling. The antineoplastic activity of HDACi is unquestionable. But studies have also suggested a possible role of HDACi in drug resistance acquisition, a frequently encountered obstacle in cancer treatment. A stable 3-fold Vorinostat-resistant HCT(1)(1)6/VOR subline was generated by stepwise exposures of the HCT(1)(1)6 colon tumor cell line to increasing concentrations of Vorinostat. This acquired Vorinostat-resistance is irreversible and is MDR-independent, and it thus differs from that reported for the HDACi Romidepsin. This acquired Vorinostat-resistance correlates with the loss of molecular responses typically seen with HDACi. These include the loss of acetylation of the histones H2A, H2B, H3, H4; the loss of G2/M checkpoint activation; and the loss of caspase 3- and caspase 7-dependent apoptosis. However, it doe s neither correlate with altered expression of HDACs, altered HDAC or histone acetyltransferease activities, altered acetylation of non-histone proteins (tubulin, HSP90, p53), increased expression of the ROS-scavenger thioredoxin, nor are alterations in the expression of proteins promoting proliferation and survival (cyclins, survivin, Bcl-2, Bcl-xL) or promoting growth inhibition and apoptosis (p2(1), p27, Bax, Bak) detected. The HCT(1)(1)6/VOR subline is cross-resistant to the hydroxamate-class (LBH589 and JNJ2648(1)585) and to the aliphatic acid-class (Valproate) HDACi, but retains responsiveness to the benzamide-class (MGCD0(1)03) and the cyclic peptide-class (Romidepsin) HDACi, and to "classic" chemotherapeutics such as Docetaxel and Doxorubicin. These results provide evidence for the potential of Vorinostat to cause acquisition of HDACi resistance in HCT(1)(1)6 colon tumor cells.

74

A novel interaction partner of the mismatch repair proteins MLH1 and PMS2 and its role in DNA repair

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Replication is an error-prone process, especially if the template DNA has been damaged by certain exogenous or endogenous agents. Mismatches and insertions or deletions that arise as errors of DNA polymerases are corrected by the postreplicative mismatch repair (MMR) system, which improves the fidelity of replication by up to three orders of magnitude. Given its importance in the maintenance of genomic stability, it is hardly surprising that MMR defects predispose to cancer. To gain further insights into the functions of mismatch repair proteins in human cells, we searched for novel partners of MMR proteins by tandem affinity purification. Among the strongest interactors of the MMR proteins MLH1 and PMS2 we identified ICE114, a protein of unknown function. Bioinformatic analyses revealed that ICE114 contains a zinc finger motif and may belong to the PD-(D/E)XK superfamily of endonucleases, the other members of which are enzymes involved in DNA repair, recombination and replication. This indication for a role of ICE114 in DNA repair could be supported by recent data from our laboratory. There, knock-out of ICE114 in DT40 cells, an avian B lymphocyte cell line, and in C. elegans, showed increased sensitivity to DNA cross-linking agents such as mitomycin C (MMC) and cisplatin. The repair of DNA cross-links seems to involve several processes such as nucleotide excision repair, homologous recombination, translesion synthesis and the Fanconi Anemia pathway. We want to learn whether ICE114 plays a role in this complex repair machinery and in which step it is involved.

75

Cytoplasmic delivery of cytochrome c via cell-penetrating peptides and via liposomes: a novel approach to induce apoptosis in tumor cells?

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Conventional, systemic chemotherapy is until today an indispensable therapeutic option for the treatment of many malignancies, but drawbacks such as the adverse side effect profiles in consequence of the high toxicity are apparent. Some of these drawbacks are directly linked to the nature of the therapeutic drug itself, while others are linked to the still limited efficiency of drug delivery and the limited tumor cell-specific delivery of therapeutically active drugs. Cytochrome c is an endogenous protein and part of the mitochondrial respiratory system but it is also critical for the intrinsic apoptotic pathway upon pro-apoptotic stimuli. This study's aim is to deliver exogenous cytochrome c via the cell-penetrating peptide (CPP) antennapedia (Antp) or via liposomes into the cytoplasm of HeLa tumor cells without the need of additional apoptotic stimuli. We found that Antp-cytochrome c more efficiently reduces cell survival and induces apoptosis to a larger extent than its ap optosis-irrelevant negative control Antp-myoglobin and the respective free compounds. This Antp-cytochrome c-induced apoptosis was almost completely abolished by the z-VAD-fmk pan-Caspase inhibitor. Cytochrome c encapsulated in liposomes made of pH-sensitive lipids reduced survival more efficiently (2.5-fold) and resulted in cleavage of the caspases-3 and -7 and of PARP-1, as compared to the respective "empty" liposomes. Taken together, apoptosis can be activated through the cytoplasmic delivery of exogenous cytochrome c via CPP or liposomes. These in vitro results seem promising in the development of a anticancer strategy that may lead to the reduction of the severe adverse side effects often seen with conventional chemotherapies.

76

Inhibition of mitochondrial Na+/Ca2+ exchange abolishes aberrant activation of protein kinase B/Akt and impairs melanoma cell survival

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The aggressive growth of malignant melanoma and its resistance to apoptosis still remains a challenge for clinical and experimental oncology. Many studies demonstrated the significance of calcium (Ca2+) signaling for melanoma malignancy. We have recently reported that the augmented store-operated Ca2+ permeation (SOC) supports aberrant activation of protein kinase B/Akt (PKB) in melanoma cells and promotes their survival (1). Mitochondria are important modulators of SOC function (2); however, the efficacy of agents targeting mitochondrial Ca2+ against melanoma has yet not been tested. Our present findings demonstrate that malignant and apoptosis-resistant clones of B16BL6 murine melanoma expressing high basal PKB activity exhibited augmented SOC function and mitochondrial Ca2+ influx/efflux rates. In contrast, non-malignant and apoptosis-susceptible clones of this tumor expressing low levels of active PKB exhibited diminished SOC function and barely detectable mitochondrial C a2+ fluxes. The specific inhibitor of mitochondrial Na+/Ca2+ exchange, benzothiazepin CGP-37157, applied to malignant B16BL6 cells effectively reduced mitochondrial Ca2+ efflux in a dose-dependent manner, decreased Ca2+ content of intracellular stores and attenuated SOC-mediated Ca2+ cell permeation. This was accompanied by inhibition of PKB activity, retarded in vitro cell growth and increased susceptibility of cells to apoptosis. Our data indicate the contribution of mitochondrial Ca2+ shuttling to deranged pro-survival signaling in melanoma cells and address the potential implication of CGP-37157 or related compounds against this frequently fatal type of cancer.

(1) Fedida-Metula S., Elhyany S., Tsory S., SegalS., Hershfinkel M., Sekler I. and Fishman D. (2008) Targeting lipid rafts inhibits Protein Kinase B by disrupting calcium homeostasis and attenuates malignant properties of melanoma cells. Carcinogenesis 29, 1546 - 1554

(2) Valero R.A., Senovilla L., Núñez L. and Villalobos C. (2008) The role of mitochondrial potential in control of calcium signals involved in cell proliferation. Cell Calcium. 44, 259-269.

77

Expression, epigenetic regulation, and immunogenicity of cancer–testis antigens in chronic myeloid leukemia (CML)

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Expression of cancer-testis antigens is characteristically restricted to cancer and the human germline and their distinctive immunogenicity makes them attractive targets in immunotherapeutic settings, e.g. in patients with CML showing imatinib-resistance or minimal residual disease. In a first step, we evaluated the expression of 30 CT antigens in 10 CML cell lines by RT-PCR. While we found that 9 antigens were expressed in the BM of healthy donors, 16 of the remaining antigens were expressed in at least one untreated CML cell line, with PRAME showing the most frequent expression (N=8). Treatment with 5'-Aza-2'-Deoxycytidine led to a two-fold increase in the average number of CT antigens expressed per cell line while treatment with Trichostatine showed an increase of only 17%. Investigating the expression of 15 promising candidates in BM and blood samples from 84 patients with CML by RT-PCR, PRAME was found in 32.1% of the samples and expression correlated significantly with stage of disease (p=0.02) and blast cell count (r=0.38; p=0.01). Sporadic expression was found for BAGE2 (N=2) and SLLP1 (N=1). Protein expression was confirmed by Western Blot. Finally, antibody-mediated immune responses were evaluated in the serum of 45 patients with CML for antibodies against 15 CT antigens by ELISA. While none of the patients showed PRAME-specific serological immune responses, one patient who had not expressed NY-ESO-1 on the RNA or on the protein level showed a significant antibody response against this antigen, possibly indicating spontaneous immunity leading to the eradication of CML clones expressing this antigen in vivo.

78

Cutaneous squamous cell carcinoma progression is associated with increased inflammation: Immunosuppression reduces CD123+ and FOXP+ cells

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The immune system fights atypical keratinocytes in the progression to squamous cell carcinoma of the skin (SCC). Drug-induced immunosuppression in organ transplant recipients (OTR) dramatically increases cutaneous carcinogenesis 60- to 100-fold. We analyzed local inflammation in the paired biopsies of intraepithelial and invasive SCC in 43 immunocompetent patients and 42 OTRs. We studied peritumoral SCC inflammatory infiltrate assessing diameter, density and phenotype (CD3, 4, 8, FOXP3, CD123, STAT1) by immunohistochemistry. Immunocompetent patients' lesions were compared to OTRs' lesions. Considerable inflammation was observed in all intraepithelial SCC (inflammatory infiltrate diameter 2.80 mm ±2.21 immunocompetent pts, 2.15 mm ±2.95 OTRs). Inflammation was more pronounced in invasive SCC of immunocompetent patients (4.60 ±4.67 mm) and OTRs (3.30 ±5.90 mm) respectively ($p<0.005$). The density of peritumoral inflammatory infiltrates increased from intraepithelial to invasive SCC ($p=0.005$). Compared to immunocompetent patients, OTRs show a lower density of peritumoral inflammatory infiltrate ($p=0.041$). OTRs also show reduced CD3+ and CD8+ cell proportions in intraepithelial SCC ($p=0.025$ and 0.027, respectively). FOXP3+ cell proportions in OTRs' invasive SCC are markedly diminished ($p=0.048$). CD123+ cells increase in the progression from intraepithelial to invasive SCC in immunocompetent patients ($p=0.040$). CD123+ cells are reduced in all SCC of OTRs ($p=0.036$). Intraepithelial SCC is accompanied by pronounced inflammation both in immunocompetent patients and OTRs. Invasive SCC is associated with further increased inflammation overall. Here, OTRs show compromised quantity and quality of inflammation, in particular reduced proportions of FOXP3+ regulatory T cells and CD123+ pDCs. This distinct inflammatory infiltrate may contribute to the CD123+ increased cutaneous carcinogenesis and more aggressive behavior CD123+ of SCC in OTRs.

79

Inactivation of the protein tyrosine phosphatase receptor R (PTPRR) by gene promoter hypermethylation is a common and early event in colorectal carcinogenesis.

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Background. Tyrosine phosphorylation, regulated by protein tyrosine phosphatases (PTPs) and kinases, is important in signaling pathways underlying tumorigenesis. In human cells, there are approximately 100 PTP genes encoding proteins that often elicit tumor suppressor functions. Indeed, several mutations in PTP genes were identified in a mutational screening of colorectal cancers (1), and epigenetic alterations of a few PTP genes have been reported in these and other cancers.
Methods. We have recently characterized the transcriptome of colorectal neoplasms and cell lines (2,3).These studies provided us with a list of putative targets of epigenetic silencing whose promoter methylation status was evaluated by bisulfite restriction analysis and sequencing.
Results. Since the early stages of transformation, the expression of a member of the PTP superfamily, PTPRR, was frequently found to be silenced in colorectal neoplasms (compared with normal mucosa samples). In particular, 85% of adenomatous polypoid lesions, 78% of adenomatous nonpolypoid lesions, 81% of colorectal cancers, and 14 out of 15 colon cancer cell lines showed an extensive methylation of the PTPRR promoter which was associated with the lack of expression of its transcript. This is the first study showing an epigenetic alteration of this member of the PTP superfamily which was not found to be mutated in the above mentioned study1.
Conclusions. Because of the crucial roles of PTPs in regulating cell growth and differentiation, mitotic cycle, and oncogenic transformation, in vitro and in vivo studies are in progress in our laboratory to evaluate the relevance of the PTPRR gene silencing in colorectal carcinogenesis.

1. Wang et al Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science. 2004 May 21;304(5674):1164-6.
2. Di Pietro et al Defective DNA mismatch repair determines a characteristic transcriptional profile in proximal colon cancers Gastroenterology. 2005 Sep;129(3):1047-59.
3. Sabates-Bellver et al Transcriptome profile of human colorectal adenomas Mol Cancer Res. 2007 Dec;5(12):1263-75.

80

Evaluation of the anti-tumor effects of new vitamin D3 analogs, BGP-13 and BGP-15.

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The roll of 1alpha,25-dihydroxyvitamin D3 [1alpha,25(OH)2D3, Calcitriol] in cancer prevention and its potential as an anti cancer therapeutic agent has been well established in variety of human tumors in vitro and in vivo. Calcipotriol is a well known vitamin D3 analogue which is considered a highly effective topical therapy available for hyperproliferative skin diseases such as psoriasis. Also, calcipotriol is known to be at least 100 times less involved than vitamin D3 in calcium (Ca2+) metabolism – therefore causing lower side effects. BGP-13 and BGP-15 are new calcipotriol-based compounds synthesized in our laboratory. We tested the effect of the administration of those compounds on the viability of different human carcinoma cell lines: LNCaP– human prostate carcinoma, MCF-7– human breast carcinoma and HT-29– human colon carcinoma, using MTT viability assay. All human carcinoma cell lines tested here displayed high susceptibility to BGP-13 and BGP-15. The cytotoxic effe ct of the two compounds on the tested cells was, in most cases, similar to the effect of vitamin D3. In addition, the molecular mechanism of cell death following treatments with the compounds was examined using a non-specific caspase inhibitor and flow-cytometry. Those results indicate that an apoptotic cell death mechanism is involved in the cytotoxic effect of the new compounds.
In conclusion, BGP-13 and BGP-15 are potentially new anti-cancer therapeutic agents. Since the two compounds originate from calcipotriol, they may potentially cause lower side effects relative to vitamin D3, while maintaining its similar significant cytotoxic effect on cancer cells and tumors.

81

Towards viral cancer therapies - the development of tropsim-modified adenovirus vectors containing pIX-barstar fusion proteins

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A major bottleneck of viral vectors in cancer gene therapy is that tropism of the native virus is typically much broader than the therapeutic indication. Furthermore, many cancer cells are not susceptible to therapeutic viruses due to low levels of virus receptors. The development of bifunctional adapter proteins that direct therapeutic adenovirus (Ad) vectors to the surface of cancer cells represents an interesting approach to increase their specificity. Here, we characterize a new adapter system for Ad vectors, which is based on the interaction of two Bacillus amyloliquefaciens proteins, barnase and barstar. Barstar is a natural inhibitor of the RNAse barnase, and binds barnase with exceptionally high affinity in the picomolar range. We have generated a series of Ad5_CMV-eGFP vectors with barstar proteins that were fused to the C-terminus of the minor capsid protein pIX protruding from the viral surface. In addition to barstar, the pIX fusion proteins contain alpha-he lical spacers of 30, 45 and 75 angstroms to increase their lengths and flexibilities, and a FLAG-tag for detection. This system will allow us to direct antibody-barnase fusion proteins to anyone of the pIX-barstar Ad5 vectors. Specifically, we plan to target Ad receptor-negative breast cancer cells expressing Her2/Neu epidermal growth factor receptors. For this, we will use Her2/Neu-specific single chain antibodies fused to barnase for binding to barstar-modified Ad5 vectors. With this approach, we hope to overcome a major limitation of therapeutic oncolytic viruses in patients, that is, their low efficacy due to low affinities to the target tissue.

82

The TSC-22 domain family may have both, an oncogenic and tumor-suppressive function in prostate cancer

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TGFbeta stimulated clone-22 (TSC-22) is the founding member of the TSC22 domain family (TSC22DF) of potential transcription factors. These genes are expressed as long and short isoforms. In Drosophila, long isoforms encoded by the TSC22DF gene bunched (bun) are positive regulators of growth and essential for fly development. By contrast, the short bun isoforms are non-essential but can act in a dominant-negative manner on the long isoforms. It is unclear whether this mechanism of growth control is conserved in humans. We have previously shown a loss of the short TSC22DF isoform TSC22D1.2 in prostate cancer (CaP). Here we investigated the expression of the complete TSC22DF in CaP cell lines and tested whether the human TSC22DF has a conserved function in growth control.
Human TSC22DF coding sequences were used for transgenic expression in Drosophila carrying a lethal bun mutation. TSC22DF expression in CaP cell lines was assessed by immunoblotting.
In Drosophila bun-mutants, transgenic expression of long but not of short human TSC22DF isoforms rescues the lethal phenotype. Human CaP cell lines PC-3 and LNCaP express two long TSC22DF isoforms, but only marginal amounts of short isoforms are expressed.
The fact that human long TSC22DF isoforms can substitute for the essential long TSC22DF isoforms in Drosophila suggests a conserved growth-promoting role for human long TSC22DF isoforms. In analogy to Drosophila, tumor-suppressive properties of TSC22D1.2 may be due to dominant-negative activity on long TSC22DF isoforms. Therefore, the loss of short isoforms may unmask an oncogenic activity of the long isoforms in prostate cancer.

83

The fate of tumor specific T cells during adoptive T cell therapy in the TRAMP prostate cancer mouse model

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Adenocarcinoma of the prostate is one of the most common malignant neoplasm in men. Unfortunately established treatments have limited success. In order to better understand the mechanisms involved in T cell tolerance induction during adoptive T cell therapy we used the TRansgenic Adenocarcinoma of the Mouse Prostate model (TRAMP). Male TRAMP mice develop SV40T antigen driven prostate cancer with striking similarities to the human disease concerning the disease progression and histology. Transfer of tumor specific CD8 T cells into TRAMP mice resulted in activation and proliferation of the transferred CD8 T cells in the prostate draining lymph nodes and subsequent infiltration of prostate tissue (Anderson et al.2007). However, prostate infiltrating tumor specific CD8 T cells quickly acquired an exhausted phenotype, with the expression of the typical cell surface markers and the loss of their capacity to proliferate or secrete IFNγ ex vivo, followed by a fast decline of the ir number in the prostate tissue. IL-2 could restore T cell function ex vivo, indicating that the lack of Th1 helper cells together with the prostate infiltrating CD11b+Gr1+ myeloid suppressor cells are responsible for the exhausted phenotype of the CD8 T cells.
Therefore combining adoptive T cell therapy with a CD11b+Gr1+ inhibiting treatment or the intra-tumoral support of Th1 cytokines has the potential to significantly improve the efficacy of CD8 adoptive T cell therapy.

Tolerization of tumor-specific T cells despite efficient initial priming in a primary murine model of prostate cancer.
Anderson MJ, Shafer-Weaver K, Greenberg NM, Hurwitz AA.
J Immunol. 2007 Feb 1;178(3):1268-76

84

Identification of Lectin-like transcript-1 as a novel mediator of glioma-associated immunosuppression

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Glioblastoma, one of the most lethal tumors, is paradigmatic for tumor-associated immunosuppression. Lectin-like transcript-1 (LLT1) is a newly identified ligand for the inhibitory NK cell receptor CD161. Here we report that glioma cells express LLT1 mRNA and protein in vitro and in vivo while expression levels in normal brain are low. LLT1 expression in human gliomas increases with the WHO grade of malignancy. We further demonstrate that transforming growth factor (TGF)-beta up-regulates the expression of LLT1 in glioma cells. siRNA-mediated down-regulation of LLT1 in LNT-229 glioma cells promotes their lysis by NK cells. Thus, LLT1 acts as a novel mediator of immune escape and contributes to the immunosuppressive properties of glioma cells.

85

Prognostic factors in well differentiated thyroid carcinomas

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Differentiated papillary and follicular thyroid cancers (DTC) have an excellent 5- and 10 year survival rate. Only 10% of these Patients cannot be cured. The goal of this study was the evaluation of morphologic criteria and immunhistochemical markers in 60 patients with DTC and adverse clinical outcome (ACO), identified by nuclear medicine departements, compared with a control group (CG) of 160 unselected DTC. All tumors were reevaluated by two pathologists. The tumor type was determined according to the 2004 WHO classification and the percentage of tall cells (TC) in the papillary carcinomas was semiquantitative measured. A tissue microarray was constructed and stained with HBME-1, Galectin-3, Thyreoglobulin, p27, PTEN, androgen receptor and VEGF. With a cut off of 10%TC per papillary carcinoma, we found 23 TC-variants in the group with the ACO of altogether 44 papillary carcinomas (52.3%) and 11 TC-variants in the CG of altogether 96 papillary carcinomas (11.5%). There were no significant differences between the two groups in the expression of HBME-1, Galectin-3 and the androgen receptor. A loss of Thyreoglobulin, p27, PTEN and VEGF is in both subtypes significantly associated (papillary and follicular) with a ACO ($P<0.001$). Summary: Already a 10% TC-quantity within a papillary carcinoma is associated with an ACO ($P<0.001$). The markers HBME-1, Galectin-3 as well as expression of the androgen receptor do not have a prognostic significance. A loss of Thyreoglobulin, p27, PTEN and VEGF is associated with an ACO. Further studies must show whether patients with such tumors benefit from an additional therapy.

86

MAPK signaling in individual living human cells

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Our cells are exposed to many stimuli, such as hormones and growth factors. These triggers dictate cell divide, move or die. Thus, cells must make decisions with a remarkable combination of speed, sensitivity and discrimination. Signal transduction pathways mediate the transmission of biochemical information from one part of the cell to another. One of the best characterized cascades is the Mitogen Activated Protein Kinase, also involved in cancer development. The third layer of this cascade, ERK, is of crucial importance as being able of nuclear translocation and activation of transcription factors involved in cell fate determination. However, little is known about the cell-cell variability in signal transduction machinery.
Here we ask what is the response of ERK at the individual cell level upon stimulation. We employ an approach for protein dynamics in individual cell based on a human cell clone, in which the ERK2 protein is tagged with YFP at its endogenous chromosomal location. We monitor ERK2's protein levels following growth factor stimulation in living cells by automated microscopy and collect data simultaneously from a large number of cells and at a high-time resolution. We quantify the biological response with respect to amplitude, duration and integrated output of ERK2. We find a wide basal variation in ERK2 nuclear levels. Despite this variability, the fold increase in nuclear levels following stimulation was remarkably constant between cells.
The present work suggests that fold rather than absolute changes in nuclear level underlie MAPK's function. This can help for drug design and clinical interventions.

Sigal A et al. (2006) Variability and memory of protein levels in human cells. Nature. Nov 30;444(7119):643-6. Epub 2006 Nov 19.

Cohen AA et al.(2008) Dynamic proteomics of individual cancer cells in response to a drug. Science. Dec 5;322(5907):1511-6. Epub 2008 Nov 20.

87

Quality of life in skin cancer patients – not only melanoma

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Skin cancer and skin cancer treatment have an impact on patients daily activities and personal relationships. Patients may suffer from disfiguring scars and - not least - skin cancer can be life threatening. It therefore has a huge impact on patients' physical and mental status. However the quality of life in patients with skin cancer other than melanoma is poorly investigated.
We therefore evaluated the tumour burden in more than 300 patients of our outpatients' clinic suffering from melanoma, epithelial skin cancer (SCC and BCC) and primary cutaneous lymphoma using the Hornheide-Questionnaire and the Dermatology Life Quality Index (DLQI).
The global score of the Hornheide Questionnaire showed remarkable differences between the three tumour groups. The highest mean value could be observed in patients with cutaneous lymphoma (mean score: 11) followed by melanoma (mean score: 7) and epithelial skin cancer (mean score: 4).
Female gender and younger age has been found to lead significantly to a decrease in quality of life. Among patients with lymphoma, sézary syndrome affected patients' quality of life most. Cutaneous B-cell lymphoma impaired life quality more than mycosis fungoides and other cutaneous T-cell lymphoma.
The DLQI score appeared to be low in all skin tumour groups. It averaged out at having no effect on patients' quality of life and was similar in all three skin tumour groups.
Hornheide Questionnaire was a more potent tool to assess quality of life in skin cancer patients than DLQI. Cutaneous lymphoma has a larger tumour burden than melanoma and epithelial skin cancers.

88

MicroRNA expression profiling in EBV-associated B-cell lymphomas

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MicroRNAs (miRNAs) are a specific class of small regulatory non-coding RNAs which play a crucial role in endogenous post-transcriptional gene regulation by repressing target mRNAs. Recent studies show relevant impact of misregulated miRNA expression for tumorgenesis. Further on, several human malignancies like Burkitt lymphoma, Hodgkin lymphoma, NPC and non-Hodgkin B-/ and T-cell lymphomas are well known to be associated with EBV. Moreover, there have been EBV-encoded miRNAs identified. The aim of the present study is therefore to evaluate the contribution of EBV to global miRNA expression, lymphomagenesis and tumor progression.

cDNA libraries (EBV+/- BCLs and tonsils) from small RNA fraction were used to deduce a profile of differentially expressed miRNAs in EBV positive lymphomas. Cellular miRNA expression level changes could be confirmed by quantitative RT-PCR.

Potentially new cellular miRNAs could be identified. A set of differentially expressed cellular miRNAs with known relation to cancer in EBV positive tumors were ruled out. For some of these miRNAs pathological subtype seems to be strongly relevant. EBV encoded miRNAs where only found to be expressed in EBV-associated B-cell lymphomas. These miRNAs where only expressed from BART clusters and account a proportion of 1.4% of all identified known miRNA reads.

Taken together, EBV seems to have a significant impact on cellular microRNA expression pattern in situ. The functional significance of this interrelation remains to be elucidated. But, this approach should provide a basis for the identification of new prognostic and/or diagnostic parameters.

89

A functional rnai screen identifies new regulators of survival and chemoresistance in human medulloblastoma cells

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Purpose. Using a high-throughput RNA interference (RNAi) screen, we sought to identify kinases that promote cell survival and chemoresistance in medulloblastoma, the most common malignant brain tumor in childhood. Method: We transfected DAOY medulloblastoma cells with a library containing 2157 unique siRNAs targeting each of the 719 human kinase genes in the presence or absence of cisplatin. Expression of selected genes was analyzed in a series comprising microarray gene expression data of primary medulloblastoma tumors and normal cerebellum.

Results. We first assessed cell viability effects induced by siRNAs in the absence of cisplatin and 33 kinases were identified as survival kinases. To identify kinases whose down-regulation promoted a drug-sensitizing phenotype, we analyzed data obtained after treatment with cisplatin and 29 kinases were identified as enhancing cell resistance to the drug treatment. A set of 4 genes (PIK3CG, MPP2, ATR and LYK5) was identified as promoting cell survival and resistance to cisplatin treatment. We further analyzed survival signaling responses following targeting of these four top selected kinases by means of RNAi or specific pharmacological inhibitors. Finally, expression of the selected kinases was analyzed in primary medulloblastoma tumor samples and potential correlations with clinical parameters were investigated.

Conclusion. RNAi targeting of specific kinases in a large-scale approach allowed identification of key regulators of cell survival and chemoresistance in medulloblastoma cell lines. Pharmacological targeting of these kinases may lead to new therapeutic strategies for this common pediatric malignancy.

90

Microsatellite instability – a common denominator in post-transplant lymphoproliferative disorders ?

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Background. Post-transplant lymphoproliferative disorders (PTLD) are a heterogeneous group of lymphoid proliferations in the context of solid organ or bone marrow transplantation. The pathobiology of this rare post-transplant complication is still poorly understood. Reasons therefore are the complexity of the lesions and the paucity of tissues available for routine diagnosis. Epstein Barr Virus (EBV) is closely involved in the pathogenesis of PTLD. Mostly, PTLD arise in response to re-activation of previously acquired EBV. However, an increasing number of PTLD develop years after transplantation, independently of EBV .
Aim. To characterize a large series of 32 EBV positive and negative PTLD, we constructed a Tissue-Micro Array. Maturation stages of PTLD and the latency type of EBV were assessed by immunohistochemistry and in situ-hybridization. Microsatellite instability (MSI) analysis for the microsatellite markers BAT-25, BAT-26, D17S250, D2S123 and D5S346 was performed.
Results. The majority (80%) of B-cell-PTLD are of post-germinal center type. EBV latency type III is the prevalent latency type in EBV positive PTLD. High microsatellite instability (MSI-H) and/or loss of heterocygosity (LOH) were found in 20% (3/15) of PTLD . Two out of the three cases with MSI, were EBV positive and corresponded to late-onset PTLD (26 and 56 months post transplantation).
Conclusion. PTLD show higher proportions of MSI, particularly of MSI-H and LOH, compared to Non-Hodgkin lymphomas in immunocompetent patients. Our results suggest an alternative mechanisms in the development of late-onset (>12 months post transplantation) and EBV associated PTLD. Further analysis of MSI in PTLD is currently under way.

91

Emergence of adaptive systems upon in vivo oncogenic Pten loss in the prostate

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Prostate Cancer is a common disease among men: Autopsy series revealed that 64 % of men in their 60s harbor small carcinomas. These lesions are in general characterized by events indicating deregulations along the PI3 kinase-PKB pathway. Importantly, the oncogenic activation of the PKB pathway is causally linked with the progression of the tumor from the localized to the metastatic state (1). However, even tough deregulated PKB signals are present shortly after tumor initiation and play a causal role in cancer progression, prostate tumors rarely grow or spread to distant sites and remain mostly clinically irrelevant. This is due to complex adaptive systems that successfully contain oncogenic signals and tumoral growth, e.g. through the activation of tumor suppressors such as p53.
We hypothesize that the progression of prostate cancers from common early lesions to clinically relevant carcinomas are caused by the failure of defined adaptive feedback systems to contain such tumoral growth. The studies of such systems are thus relevant for the understanding of prostate cancer progression and might reveal novel therapeutical targets.
In this study we describe the emergence of novel and defined adaptive signatures in the N-linked glycoproteome of a mouse model of prostate cancer following Pten deletion and constitutive PKB activation. This approach revealed interesting adaptive responses in compartments particularly relevant for targeted therapy such as the plasma membrane, the extracellular space and the secretory pathway.

(1) Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. N Engl J Med. 2003 Jul 24;349(4):366-81.

92

First report of the genomic evolution of a hormone-refractory human prostate cancer

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Aims. Intratumoral clonal heterogeneity and complexity are frequent in prostate cancer and can mask cell populations. Aim of this study was to analyze the genomic clonal evolution of a prostate tumor and its development to hormonal independence over the whole course of disease by using standard pathological as well as high definition genomic profiling technologies.
Methods. Three frozen carcinoma specimens from the same patient were collected over a time period of eight years (2000, 2007, 2008). Using a multistep approach involving high-end flow-sorting based on degree of aneuploidy, distinct clonal populations were isolated and analyzed for genomic aberrations by high-resolution array-CGH using the 244k Agilent microarrays.
Results. We show the genomic evolution of a clonal population of hormone-sensitive prostate cancer (2000) into two neoplastic (one diploid and one aneuploid) hormone-independent populations after orchiectomy accompanied by androgen receptor gene amplification and novel undescribed genomic aberrations. Interestingly, only the aneuploid population, but not the diploid one responded to secondary anti-androgenic therapy in 2008.
Conclusions. High resolution profiling of distinct clonal populations of a prostate cancer over time reveals new insights into the clonal evolution of the tumor cells and may pinpoint to new genomic-driven therapeutic strategies against prostate cancer.

93

Cell surface proteomics reveals new protein markers for the discrimination of malignant pleural mesothelioma from lung Adenocarcinoma

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Introduction. The diagnosis of malignant pleural mesothelioma (MPM) is still a problem for clinicians as well as for the pathologists. The histopathological approach is complicated by a broad differential diagnosis. Currently, panels of histopathological marker are needed to discriminate MPM from anatomically related malignancies like lung adenocarcinoma. Using mass-spectrometry we set out to identify cell surface protein patterns discriminatory for MPM versus lung adenocarcinoma.
Methods. We investigated the cell surface subproteome of one epithelial MPM cell lines and one adenocarcinoma cell lines via the Cell Surface Capturing (CSC) technology. Relative quantification of identified cell surface proteins was achieved by Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC). Differentially expressed proteins were confirmed at the mRNA level on a collection of MPM and adenocarcinoma cell lines. Candidate proteins were validated by IHC staining on cell lines and patient samples.
Results. Over 130 bona fide cell surface glycoproteins were identified and quantified via CSC technology, among them 37 CD annotated proteins. 62 cell surface glycoproteins were found to be differentially expressed between MPM and adenocarcinoma. RT-PCR analysis on 15 MPM and 6 adenocarcinoma cell lines revealed two glycoproteins as potentially discrimination markers. One out of the two cell surface glycoproteins were confirmed by IHC on patient tissues using a commercially available antibody.
Conclusion. By using the CSC technology in a quantitative proteomics approach we were able to identify cell surface glycoproteins differentially expressed between mesothelioma and adenocarcinoma cells. Two selected proteins indicate the potential of discriminating MPM from adenocarcinoma.

94

Limited influence of individual cytokines for vaccine induced CD8+ T cell response

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In the present study, we assessed the influence of cytokines on the induction of a vaccine induced CD8+ T cell response. So far, most studies addressing the role of cytokines for CD8-responses used viral models. These studies are hampered by the complexity of events triggered by viral replication, including the activation of a multitude of innate stimuli, owing to the complex structure of microorganisms that signal via different pathogen association molecular patterns (PAMs) and massive cell death usually occurring during infection.

Virus-like particles (VLPs) consist of multiple copies of certain structural viral proteins, which self-reassemble into spherical structures resulting in a viral shell devoid of genetic information required for viral replication. Vaccines on the basis of VLPs are very efficient in inducing CD8+ T cell responses when administered together with toll-like receptor-ligands, which are effective activators of APC. We were able to dissect the role of cytokines for the support of antigen-specific T cell expansion in the context of immuization with VLPs alone or VLPs applied together with distinct TLR stimuli by using mice deficient for Th1 promoting cytokines or their respective receptors.
To our surprise, the only cytokines that played a major role in CD8+ T cell-activation, expansion and memory establishment were type I interferons. Defective signalling in IL-12, IL-23 or IFNg had no major effect on establishment of functional CD8-responses and might therefore be considered redundant for the initiation of CD8+ responses.

95

Prognostic significance of integrin b1 and L1 cell adhesion molecule in non-small cell lung cancer

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Background. Epithelial-mesenchymal transition (EMT) might be involved in tumour progression and metastasis. Integrin and epidermal growth factor receptor (EGFR) signalling can induce EMT and downregulate E-cadherin. Periostin is an EMT indicator and modulates integrin/EGFR crosstalk. The cell adhesion molecule L1 (L1-CAM) mediates EMT in carcinoma cells by modulating E-cadherin and integrin b1. We investigated the correlation of these proteins with clinico-pathologic parameters and overall survival in non-small cell lung cancer (NSCLC).
Methods. Expression of stromal periostin, membranous integrin b1, L1-CAM, EGFR, and E-cadherin was immunohistochemically analysed in a tissue microarray of tumour tissue of 535 NSCLC patients. Associations of protein expressions with clinico-pathologic parameters were calculated by chi-squared tests, overall survival by the Kaplan-Meier method.
Results. Increased expression of stromal periostin was found in 61%, of membranous integrin b1, EGFR, E-cadherin and L1-CAM in 44%, 23%, 55% and 2% of the tumours, respectively. Increased stromal periostin was significantly associated with increased membranous integrin b1 and EGFR (p-values <0.001) and as a trend with increased membranous L1-CAM (p-value 0.068) in the tumour cells. Increased membranous integrin b1 and L1-CAM were significantly correlated with decreased survival on univariate analysis and found to be independent prognostic factors on multivariate cox regression hazard models (including pT, pN and grade, all p-values <0.05).
Conclusions. Integrin b1 and L1-CAM could be useful prognostic markers in NSCLC. Whether the stromal EMT protein periostin causally induces increased expression of membranous integrin b1 and EGFR in NSCLC, has to be clarified in further functional studies.

Mimeault M and Batra SK. Interplay of distinct growth factors during epithelial-mesenchymal transition of cancer progenitor cells and molecular targeting as novel cancer therapies. Annals of Oncology 2007; 18:1605-1619

Hehlgans S, Haase M, and Cordes N. Signalling via integrins: Implication for cell survival and anticancer strategies. Biochimica et Biophysica Acta 2007; 1775: 163-180

Soltermann A, Tischler V, Arbogast S, Braun J, Probst-Hensch N, Weder W, Moch H, and Kristiansen G. Prognostic significance of epithelial-mesenchymal and mesenchymal-epithelial transition protein expression in non-small cell lung cancer. Clin Cancer Res. 2008; 14:7430-7

96

Identification and validation of differentially expressed miRNAs of prostate cancer

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Aims. We aimed to identify differentially expressed microRNAs in prostate cancer by comparing miRNA expression in tumor and normal adjacent tissue.
Methods. 24 pairs of fresh frozen matched tumor and normal adjacent tissue were analysed using human miRNA-microarrays encoding probes for 470 human and 64 human viral microRNAs from the Sanger database v9.1. Regulated miRNAs were further validated in qRT-PCR using TaqMan Probes in 76 pairs of matched normal and tumor tissue. Data were normalized to hsa-miR-130b.
Results. Fourteen miRNAs with expression changes greater 1.5 fold in prostate cancer were found. Unsupervised cluster analysis displayed good discrimination between normal and tumor samples. These differentially expressed miRNAs and four previously described miRNAs were further validated by qRT-PCR. Ten miRNAs showed a significantly decreased expression in tumor tissue with expression changes ranging from 1.33 to 3.74 fold. Five miRNAs were significantly increased with a 1.23 to 1.61 fold higher expression in tumor tissue.
Conclusions. Prostate cancer is characterized by significant miRNA expression changes that clearly discriminate between tumor and normal tissue and that might represent new therapy targets. Further studies validating these findings in larger, clinically characterized tumor cohorts are underway.

97

Peripheral and local tumor specific T-cell responses in renal cell carcinoma

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The prognosis of patients with renal cell carcinoma (RCC) is poor as 30% of newly discovered RCCs are already in a metastatic stage and RCC are highly resistant to chemo- and radiotherapy. Immunological treatment modalities for patients with RCC are currently hampered by the still incomplete view on immune mechanisms involved in RCC rejection. Local immune responses are often compromised and there is evidence that the tumor environment itself contributes to this immunosuppression. We aim to characterize those immunosuppressive mechanisms as specific interference presumably will enhance spontaneous and vaccine-induced tumor-specific immunity and thus improve tumor control. In a first panel of sections from RCC, we detected infiltrating effector cells (perforin+) as well as regulatory T cells (FoxP3+), which supports the presumed local immunosuppression. We screened a collection of RCC cDNAs for the expression of different Cancer Testis (CT-) and Tumor Associated- Antigens (TAAs) and we will analyze the CD8+ T-cell response within PBMCs and TILs towards those antigens expressed in the tumor using stimulation with known HLA-A2-restricted peptide epitopes. Furthermore, we will systematically block the action of immunosuppressive pathways such as Tregs, TGF-b, IL-10, CTLA-4, PD-1, PD-L1, BTLA and HVEM during stimulation to identify mechanisms involved in local subversion of the immune response in RCC.

98

The role of COX-2/PGE2 in Helicobacter felis associated gastric carcinogenesis in C57Bl6 mice

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COX-2 protein expression and PGE2 production are significantly induced in the Helicobacter infected human gastric mucosa and in a variety of gastrointestinal cancers (1). COX-2 has been proposed as a target for anti-cancer therapy; however, the results of COX-2/PGE2 inhibition and its role in the development of gastric cancer are controversial and depend on the utilized models.

In our mouse model of H.felis infection, we observe gastric cancer precursor lesions 3 months post infection. To clarify the role of COX-2/PGE2 pathways in this context, we inhibited the function of COX-2 during infection with Celecoxib and, on the other hand, administered exogenous PGE2 to infected mice.

Our results show that COX-2 inhibition in vivo aggravated the development of early epithelial changes, whereas intraperitoneal administration of PGE2 completely abrogated both gastritis and epithelial pathology and reversed pre-existing precancerous lesions. In addition, PGE2 administration reduced pro-inflammatory transcripts (eg. MIP-2) expression in the murine mucosa as well as in epithelial cells in vitro. PGE2 treatment further diminished ex vivo splenocyte proliferation and migration ability after stimulation with Helicobacter lysate or conditioned media respectively. Our results suggest an immuno-suppressive function for PGE2 in H.felis infected mice, acting on the epithelium and immune cells thereby inhibiting the onset of precancerous lesions.

99

The transcriptome of nonpolypoid colorectal lesions

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Background. Colorectal cancers develop from polypoid or nonpolypoid (usually slightly elevated, but <2.5mm from the adjacent mucosa) precancerous lesions. Microscopically, while the former lesions display only adenomatous histology, nonpolypoid lesions can present either adenomatous or serrated features. The potential of malignant transformation of nonpolypoid lesions, compared with that of polypoid lesions, is a matter of debate. We intended to address this issue by investigating their global gene expression profiles.

Methods. Exon arrays (Affymetrix) containing oligonucleotides recognizing all the exons of human genes, were used to compare the transcriptomes of 25 nonpolypoid and 17 polypoid lesions excised from the proximal colon, and their normal mucosa counterparts.

Results. Data analysis showed a clear separation between all precancerous lesions and normal mucosa samples. Nonpolypoid and polypoid lesions could also be easily segregated, with the former group displayingm less dramatic transcriptomic changes. While the Wnt signaling was similarly dysregulated in both groups of lesions, nonpolypoid lesions did not display extensive expression changes of genes involved in cell cycle regulation which were found in polypoid lesions. Within the group of nonpolypoid lesions, canonical correspondence analysis allowed the identification of clinical/pathologic variables (i.e., patient age, lesion diameter and histology) that were clearly associated with distinct clusters of expression profiles. In particular, serrated lesions clustered differently from nonpolypoid villous adenomas. Conclusions. Although our data suggest that nonpolypoid lesions might have a lower potential of transformation (compared with polypoid lesions), their size and histology, along with the age of their carriers, should be carefully considered in clinical management.

100

Calretinin: An important factor in designing drug therapy in colon cancer?

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Colon cancer is the second leading death-causing cancer in Western countries. Several proteins including transcription factors are up- regulated or down-regulated, which are linked to colon cancer progression. One of the proteins up-regulated in poorly differentiated colon cancers is the calcium-binding protein (CaBP) calretinin (CR), which is not expressed in normal colon epithelial cells. CR is a CaBP of the EF- hand family, which is expressed also in mesothelioma of the epithelial and mixed type. CR was found to be up-regulated in vitro in 5-fluorouracil (5-FU) treated colon cancer cells, which was discussed as a factor in making cancer cells more resistant to 5-FU treatment. We first checked for cytotoxic effects of 5-FU and an inducer of differentiation, sodium butyrate (NaBt) in the colon cancer cell lines HT-29, WiDr (both CR-positive) and CaCo-2 (CR-negative). Compared to CaCo-2 cells, CR-positive cells were more resistant to either 5-FU or NaBt treatment. Furthermore HT-29 cells expressing the highest CR levels were more resistant to a combination treatment of 5-FU and NaBt in comparison to WiDr cells. Also transient increase in CR expression was more pronounced in HT-29 cells than in WiDr cells after combination treatment. Our results support the hypothesis that CR plays a role in the resistance mechanism to 5-FU and NaBt treatment. Our data indicate that CR may be an important factor to be considered in developing new drugs or designing drug therapies in colon cancer treatment.

101

S6K1-mediated disassembly of mitochondrial URI/PP1gamma complexes activates a PP1gamma-Dependent negative feedback program that counters S6K1 survival signaling

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Ribosomal S6 Kinase 1 (S6K1) acts to integrate nutrient and growth factor signals to promote cell growth as well as cell survival as a mitochondria-tethered protein kinase that phosphorylates and inactivates the pro-apoptotic molecule BAD. Here we report that the prefoldin chaperone URI represents a novel substrate of S6K1 in vivo. In growth factor-deprived or rapamycin-treated cells, URI forms stable complexes with protein phosphatase (PP)1gamma at mitochondria thereby inhibiting phosphatase activity. Growth factor stimulation induces disassembly of URI/PP1gamma complexes through S6K1-mediated phosphorylation of URI at serine 371 and the subsequent activation of a PP1gamma-dependent negative feedback program, resulting in diminished S6K1 activity and BAD phosphorylation and enhanced sensitivity of cells to BAD-dependent apoptosis. These findings establish that URI and PP1gamma are integral components of a previously unrecognized S6K1-regulated mitochondrial pathway dedicated , at least in part, to oppose sustained S6K1 survival signaling, thereby ensuring that the mitochondrial threshold for apoptosis is set in accord with the availability of nutrients and growth factors.

Djouder N et al.
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102

The dual role of CD27 signaling in tumor surveillance and promotion

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Introduction. CD27 is a member of tumor necrosis factor receptor (TNFR) family and expressed on T cells, activated B cells and some NK cells. Its ligand CD70 is tightly controlled. CD27 activation results in NFkB activation, enhances TCR-mediated expansion and survival and increases effector function. In contrast some tumors in human express CD70 and its expression is thought to be linked to improved tumor growth. Why in some situations CD27 ligation improves T cell responses and tumor control whereas in other situations it has detrimental consequences for the host is unknown.
Results. We analyzed anti-tumoral immune responses and tumor control in a CD27-/- mouse model. We found that the tumor-specific CTL response after injection of tumor cells as single cell suspension is reduced in CD27-/- mice. In contrast tumor growth of transferred tumor fragments was reduced in CD27-/- mice when compared with WT mice. Similarly, spontaneous tumor development and growth after MCA treatment was reduced. CD70 was highly expressed on tumor infiltrating DCs, macrophages, CD4+ and CD8+ T cells. Depletion of CD4+ T cells resulted in rejection of MC57 tumor. Adoptive transfer of CD27+ CD4+ T cells in CD27-/- mice enhanced tumor growth.
Conclusions.
1. CD70-CD27 signaling improves anti-tumoral CTL immune response to tumor cells injected as single cell suspension.
2. In contrast chronic CD27-CD70 signaling misdirects the immune system to an impaired anti-tumoral immune response. Possible mechanisms are an enhanced generation and/or induction of CD25+ FoxP3+ CD4+ regulatory T cells.
3. Blocking CD27-CD70 signaling may improve a novel therapeutical strategy to improve tumor rejection.

103

VHL loss causes spindle mis-orientation and chromosome instability

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Error-free mitosis depends on fidelity-monitoring checkpoint systems that ensure correct temporal and spatial coordination of the process of chromosome segregation by the microtubule (MT) spindle apparatus. Defects in these checkpoint systems can lead to genomic instability, an important aspect of tumourigenesis. The von Hippel-Lindau tumour suppressor protein pVHL is inactivated in several human tumours including clear cell renal cell carcinoma (ccRCC) and is thought to exert its tumour suppressor function, in part, by functionally associating with interphase and ciliary MTs 1, 2. Here we show that pVHL localizes to the mitotic spindle in primary and transformed mammalian cells and that its functional inactivation provokes spindle mis-orientation, spindle checkpoint weakening and chromosomal instability. Spindle mis-orientation is, in VHL-deficient cells, linked to unstable astral MTs and rescued by restoration of wild-type pVHL function but not naturally-occurring VHL disea se mutants that are defective in MT stabilization. Impaired spindle checkpoint function and chromosomal instability is the result of reduced Mad2 levels actuated by VHL inactivation. Consequently, cells fail to fully enrich Mad2 on unattached kinetochores upon spindle checkpoint activation, display chromosome mis-segregation in anaphase and mitotic slippage in the presence of a spindle inhibitor, and develop aneuploidy. Re-expression of Mad2 in VHL-defective cells reverts these effects. An association between VHL inactivation, reduced Mad2 levels and increased aneuploidy was also found in human ccRCC. Together, these observations reveal previously unrecognized functions of pVHL in promoting proper spindle orientation, error-free mitosis and chromosomal stability that likely contribute to tumour suppression in VHL-associated neoplasias.

1. Hergovich, A., Lisztwan, J., Barry, R., Ballschmieter, P. & Krek, W. Regulation of microtubule stability by the von Hippel-Lindau tumour suppressor protein pVHL. Nat Cell Biol 5, 64-70 (2003).
2. Thoma, C. R. et al. pVHL and GSK3beta are components of a primary cilium-maintenance signalling network. Nat Cell Biol 9, 588-95 (2007).

104

Differential regulation of nuclear orphan receptors 4A by adenosine receptors

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Adenosine is a nucleoside whose concentration increases during metabolic stress. It regulates several cellular pathways through four G-coupled receptors (AR) A1-AR ¬, A2A-AR ¬, A2B-AR and A3-AR. Increased levels of adenosine are found in tumor microenviroments and it has been suggested that this nucleoside could play a regulatory role in tumor biology. Adenosine can, in fact, induce both proliferation and apoptosis, stimulate angiogenesis and regulate immune responses to tumors.
In this study we first identified adenosine receptor subtype-specific factors by a genome-wide transcriptional approach in the human mast cell-line 1 (HMC-1). By differential profile analysis, we detected that nuclear orphan receptors 4A 2 and 3 (NR4A2 and NR4A3) rapidly react upon treatment with the adenosine analog NECA. NR4As are transcription factors that have been linked to cell cycle regulation and carcinogenesis. A more detailed analysis revealed that these factors are upregulated upon A2B-AR and A3-AR engagement, whereas selective A2A-AR activation failed to induce them. In fact, this later receptor sub-type appears to counteract NR4A2 and NR4A3 induction by NECA. We further detected an increment of these two factors in the nucleus upon NECA treatment, which was accompanied by an increase in their activity. In contrast, A2A-AR activation failed to stimulate NR4A2 and NR4A3 transcriptional activity. Based on these results, we propose that NR4A2 and NR4A3 may represent relevant downstream effectors of adenosine in the context of tumor biology, and that regulation of these transcription factors by AR subtypes could represent a novel therapeutic strategy against cancer.

105

An unexpected role for caspase-10 in death receptor-induced neuroblastoma 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). Both caspases-8 and –10 are frequently downregulated in aggressive NB cells, and silencing of caspase-8 expression was shown to be responsible for their resistance to TRAIL. We have previously demonstrated that stable re-expression of caspase-8 fully restored TRAIL sensitivity in the caspase-8/-10 negative IGRN-91 cell line. In contrast, the role of caspase-10 and its ability to substitute for caspase-8 in death receptor-induced apoptosis is still controversial. Here, we analysed the particular contribution of caspase-10 in apoptosis initiation in NB cells.
In contrast to caspase-8, stable re-expression of caspase-10 in the IGR-N91 cells was unable to restore TRAIL sensitivity. Reverse experiments were performed in TRAIL sensitive caspases-8/-10 positive NB cells using RNA interference. Caspase-8 silencing resulted in complete resistance to TRAIL, indicating that caspase-10 on its own was unable to substitute for caspase-8 to activate downstream caspases, Bid and ultimately apoptosis. Interestingly, caspase-10 silencing enhanced NB cells sensitivity to TRAIL and resensitise SH-EP NB cells to FAS-induced apoptosis.
In conclusion, caspase-10 is not able to substitute caspase-8 in NB cells to initiate a full apoptotic cascade in response to TRAIL. Thus, NB cells resistance to TRAIL is not caused by downregulation of caspase-10 expression, in contrast to caspase-8. Moreover, caspase-10 silencing in caspase-8 positive NB cells increases their sensitivity to TRAIL and FAS-L, indicating that caspase-10 plays an unexpected anti-apoptotic role in the initiation of death receptor-mediated apoptosis.

106

Functional characterization of matrix metalloproteinase 1 (MMP-1) expression modified osteosarcoma cell lines

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Osteosarcoma is the most frequent primary malignant tumor of bone typically affecting children and young adults. It is associated with a very poor prognosis for patients with metastasis at diagnosis. Proteolytic activity is important at multiple stages of metastasis. We have shown that MMP-1 is strongly upregulated in highly metastatic 143-B osteosarcoma cells in comparison to the parental HOS cells. In different in vitro assays we have compared MMP-1 expression modified HOS and 143-B cells with the parental cells.
HOS cells infected with a retroviral MMP-1 expression construct expressed significant amounts of the protein. In 143-B cells, infected with a MMP-1 specific siRNA construct but not with an unspecific construct, MMP-1 protein expression was strongly downregulated. Highly metastatic 143-B cells and HOS cells overexpressing MMP-1 adhere better to Collagen type I than HOS cells. MMP-1 downregulated 143-B cells showed a small, but significant reduction in cell adhesion to Collagen type I in comparison to the parental 143-B cells. In soft agar assays, 143-B cells but not HOS cells form significant amounts of large colonies. For HOS cells overexpressing MMP-1, a strong increase of large colonies were observed. For 143-B cells downregulated in MMP-1 expression, but not cells infected with the empty vector or an unspecific siRNA sequence, a decrease in the formation of large colonies was found.
Specific modifications of MMP-1 expression in osteosarcoma cells influence the functional properties of these cells for cell adhesion to Collagen type I matrices and the ability of anchorage independent growth in soft agar.

107

Crosstalk between DNA mismatch repair and chromatin assembly

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Cells are constantly exposed to endogenous and exogenous genotoxic stress, which endangers genomic stability.
Errors arising during replication are targeted either by the proofreading activity of the replicative polymerases or by the DNA mismatch repair pathway. If mismatch repair fails, the cells acquire a mutator phenotype that is most easily detected as microsatellite instability (MSI), a hallmark of hereditary non-polyposis colon cancer (HNPCC), one of the most common cancer predisposition syndromes.
Mismatch repair is thought to take place on newly-replicated, histone-free DNA. However, newly-replicated DNA is rapidly bound by histones and chromatin assembly factors, and it is likely that packaging of DNA into chromatin would inhibit MMR. To allow the repair of replication errors, we postulate that mismatch repair proteins signal to suppress chromatin assembly and thus open a time window during which mismatch repair can take place.
We found biochemical evidence for such a crosstalk, involving the mismatch repair protein MSH6 and the chromatin assembly factor CAF-1.

108

Novel transition state-based compounds of the vitamin B6-dependent ornithine decarboxylase suppress proliferation of tumor cells in particular glioma

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Creating transition-state mimics has proven to be a powerful strategy in developing inhibitors to treat malignant diseases in several cases. In the present study, structurally diverse coenzyme-substrate derivatives mimicking this type for pyridoxal 5'-phosphate-dependent human ornithine decarboxylase (hODC), a potential anticancer target, were designed, synthesized, and tested to elucidate the structural requirements for inhibition of intracellular ODC as well as of tumor cell proliferation. Structural analysis of the active site of hODC disclosed a hydrophobic pocket adjacent to the e-amino group of its substrate ornithine, which could be utilized to design potent inhibitor of hODC. Of 23 conjugates, phosphopyridoxyl-ornithine(BOC) methyl ester, phosphopyridoxyl- and pyridoxyl-L-tryptophan methyl ester (POB, pPTME and PTME, respectively) proved significantly more potent in suppression proliferation (IC50 ~ 25 µM) of glioma cells (LN229) than alpha–DL-difluoromethylornithine (DFMO), a medically used irreversible inhibitor of ODC. The inhibitory active compounds feature a hydrophobic side chain fragments and a kind of polyamine motif (-NH-(CHX)4-NH-). In addition, they induce, as polyamine analogs often do, the activity of the polyamine catabolic enzymes polyamine oxidase and spermine/spermidine N1-acetyltransferase. The dual-action mode of these compounds in LN229 cells affects the intracellular polyamine metabolism and might underlie the more favourable cell proliferation inhibition in comparison with DFMO.
A combination chemotherapy with DFMO to treat recurrent anaplastic gliomas, the most aggressive form of brain tumors with poor prognosis, looked promising. The present investigation might foster development of even better inhibitors on the basis of coenzyme-substrate mimics of hODC to target tumors like gliomas, be it as mono drug or in combination.

1 Fang Wu, et al. Structural requirements for novel coenzyme-substrate derivatives to inhibit intracellular human ornithine decarboxylase and cell proliferation. FASEB J. 2008 Oct 15. [Epub ahead of print]

2 Fang Wu, et al Inhibitory and structural studies of novel coenzyme-substrate analogs of human histidine decarboxylase. FASEB J. 2008 Mar;22(3):890-7. Epub 2007 Oct 26

3 Fang Wu, et al Transition state based-inhibitor of human ornithine decarboxylase inhibiting growth of tumor cells. Molecular Cancer Therapeutics. 2007 Jun;6(6):1831-9

109

Diagnostic cell surface protein barcodes of blood cancers

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Accurate classification of hematological malignancies is a prerequisite for correct diagnosis, prognosis and therapy. Classification of blood cancer subtypes is limited by the number of known cell surface classification markers, which are suitable for immunophenotyping. Hence, a systematic and quantitative analysis of cell surface proteins is required to identify new classification markers on blood cancer subtypes.
The mass spectrometry-based cell surface capturing (CSC) technology enables a systematic and quantitative analysis of cell surface protein expression patterns. The CSC technology comprises complementary protein tagging strategies for high affinity enrichment of peptides derived from cell surface proteins. Protein identification and quantification are accomplished by liquid chromatography coupled to tandem mass spectrometry analysis.
Our study comprises human leukemia, non-Hodgkin's lymphoma and Hodgkin's lymphoma cell lines. We identified by mass spectrometry over 1100 membrane proteins, including 224 CD annotated cell surface proteins. Differentially expressed cell surface proteins are characteristic for each blood cancer subtype and thus the protein expression pattern may be utilized, like a barcode (containing protein identities and quantities), for improved molecular classification of cancer subtypes. We identified a panel of new candidate classification markers, which we are currently investigating on a patient tissue microarray that contains over 120 distinct lymphoma cases.
Proteomic cell surface barcodes will enable an improved molecular classification of blood cancer subtypes and provide the molecular basis for predictive and preventive medicine.

Tinguely, M., Hofmann, A., Bausch-Fluck, D., Moch, H., & Wollscheid, B., Immunophenotyping without antibodies : New perspectives for lymphoma characterization. Pathologe (2008).

110

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, and c-Myc. 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 and will be complemented by radioresistant DNA synthesis and measurements of BrdU incorporation. Pulse field gel electrophoresis shows increasing DNA breakage upon oncogene overexpression. We have established DNA fibre labelling to analyse the effect of oncogene-induced hyper- and rereplication on initiation and progression of individual replication forks. Based on these results, we will visualise by electron microscopy in vivo replication intermediates from cells exhibiting replication stress and identify pathological structures. We are confident that this study will greatly improve our understanding of oncogene-in duced replication stress in early tumourigenesis.

111

Structural analysis of DNA replication across unstable repetitive sequences (TNR)

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Trinucleotide repeats (TNR) can undergo large deletions or expansions, leading to neurodegenerative diseases like Friedreich's ataxia, Huntington disease or Fragile X. Expanded TNR were shown to form in vitro unusual secondary structures and to stall DNA replication forks, but the molecular mechanisms leading in vivo to DNA replication interference and repeat expansion are still elusive.

We aim to analyse in vivo DNA replication intermediates across mammalian TNR, by infecting COS-1 cells with SV40-modified viruses, containing different numbers of GAA repeats. After ending the cloning steps, we will test experimental conditions for in vivo replication of these constructs. SV40 DNA replication intermediates will then be analysed by DNA 2D-gels and electron microscopy (EM) to reveal the possible presence of unusual DNA structures. Infecting cell lines depleted (siRNA) for specific cellular factors, we aim to reveal the role of candidate replication fork players in TNR replication and stability.

We also aim to establish a DNA-antibody able to recognize the DNA structures associated with expanded TNR. By agarose gel mobility shift, we isolated the secondary structure formed at expanded GAA repeats and confirmed, by high-resolution EM analysis, the current hypothesis of a "triplex-DNA" structure. After stabilization (crosslinking) and large-scale purification, this secondary structure will be sent to Prof. Mori's laboratory for monoclonal antibody isolation and in vivo validation in proper experimental systems (infection with expanded TNR constructs, Friedrich's Ataxia patient cell lines). Our ultimate goal is to use this read-out for a genome wide shRNA-screen of novel cellular players in TNR stability.

112

Expression and immunogenicity of cancer/testis antigen MAGE-C1/CT7 in melanoma patients

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Cancer Testis (CT) antigens represent good candidates for cancer vaccination therapy as their expression is restricted to cancer cells and germ cells of the testis. MAGE-C1/CT7 is a CT Antigen that is highly expressed in several cancers. The spontaneous occurrence of CT7-specific antibodies was previously described in multiple myeloma patients; however spontaneous CT7-specific T cell responses were not detected so far. In a retrospective study using tissue-microarrays from primary melanoma lesions, peripheral and brain metastases, we found that 78/222 (35%) of lesions expressed CT7 by immunohistochemistry (IHC). The humoral response against CT7 from a cohort of 79 melanoma patients was analysed by Western blot. A spontaneous specific antibody response was detected in 11 out of 79 patients (13%). Some of these responses were high-titered up to 1: 20´000. Furthermore CT7 specific T-cell responses were assessed from this cohort of patients. We detected MAGE-C1/CT7 specific CD4+ T-cell responses in 2 out of 9 seropositive patients and in 2 out of 12 seronegative patients. CT7 specific CD4+ T cell monoclonal populations were generated and restriction elements and epitopes defined. So far nor CD8+ T-cell responses were found.

This study demonstrates for the first time that CT7 does induce a CD4+ T cell response in cancer patients. Moreover, frequent spontaneous immune responses were found, both humoral and cellular against CT7, correlating with antigen expression in melanoma tissues as detected by IHC. Based on these results CT7 will be further explored as a potential vaccine for cancer immunotherapy in melanoma patients.

113

Interleukin-12 initiates tumor rejection independent of NK cells and adaptive immunity

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Lymphocytes and their secreted cytokines are considered to play a critical role in tumor elimination. In a variety of tumor models, Interleukin-12 (IL-12) has been shown to repress tumor growth. The tumoricidal activity of IL-12 is widely held to be mediated by the activation and polarization of NK and TH1 cells respectively. Using gene-therapy, we found drastic growth repression in vivo of B16 melanocytes constantly secreting low amounts of IL-12 (B16-IL12), while the parental B16 cells form a massive subcutaneous tumor. The usage of IL-12Rbeta2 deficient mice revealed that the tumor derived IL-12 acts on the host rather than the tumor itself and BM-chimeras showed that IL-12 engages a hematopoietic cell population. Surprisingly B16-IL12 also failed to grow subcutaneously in RAG-1-/- mice, demonstrating that neither MHC-restriction nor B and T lymphocytes are involved in IL-12-mediated tumor elimination. We could also show that NK cell depletion in RAG-1-/- mice as well as the use of IL-15Ralpha deficient mice did not render the repression of the subcutaneous tumor growth after challenge with B16-IL12 melanocytes. In summary our data clearly demonstrate that the IL-12-mediated tumor suppression acts independent of NK cells and adaptive immunity. The induced immune response does not act systemically but in a local highly efficient manner. This novel, unprecedented IL-12-mediated pathway of immune action reveals a potential therapeutic mode of IL-12 in tumor suppression.

114

Uncovering the structural determinants of DNA replication stress induced by topoisomerase inhibition

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Topoisomerase 1 (TopI) inhibition by camptothecin (CPT) has long been considered a molecular mechanism for cancer chemotherapy. CPT water-soluble derivatives Topotecan and Irinotecan are indeed FDA-approved treatments for several cancers. These molecules form a ternary complex (TopIcc) with the DNA and TopI, resulting in single stranded nicks (ss-nicks) in the DNA. A long-standing model of action of these drugs ("run-off theory") suggests that DNA replication forks would encounter these nicks and convert them into double stranded breaks (DSB), highly cytotoxic lesions, which would require recombinational repair to promote replication fork restart.
Recent reports have challenged this hypothesis, showing that Top1 inhibition leads to the accumulation of positive supercoils and that forks are actively prevented from colliding with Top1cc. We aim to structurally investigate the impact of Top1-inhibiton on DNA replication, combining a range of molecular biology and structural techniques, such as bi-dimensional gel electrophoresis in yeast, DNA combing in mammalian cells, PFGE, replication assays in Xenopus oocyte extracts and Electron Microscopy of replication forks.
Preliminary data suggest that both origin activation and replication fork progression are affected by Top1-inhibitors, but DNA synthesis seems independent upon recombinational repair, challenging the "run-off" theory. The combined EM and PFGE analysis will reveal whether ss-nicks or DSB accumulate at replication forks and whether topological stress results in unusual DNA structure. Extending our analysis to yeast mutants, siRNA-depleted mammalian cell lines and immuno-depleted Xenopus extracts, we plan to test the role of candidate factors in controlling replication fork structure and progression in response to Top1-inhibition.

115

Interaction between adult muscle precursor cells and preexisting urological cancer

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The replacement of terminally damaged organs remains a major problem in healthcare. The shortage of available donor organs and high immunosuppressive therapy morbidity lead to application of regenerative medicine for organ replacement. The use of autologous cells for organ reconstruction has the potential to overcome these shortcomings and provide replacement organs made from the patients own cells. Muscle Precursor Cells (MPCs), for muscle regeneration, are envisioned as promising cell sources capable to regenerate muscle fibers, and therefore investigated for the treatment of several muscular diseases. In Urology, it opens novel treatment possibilities including reconstruction of bladder muscles, management of sexual dysfunction and treatment of Urinary Incontinence. Functional muscle fibers decrease with age due to apoptosis and are one of the morphological bases for the higher incidence of stress urinary incontinence (SUI) in the elderly. Tissue engineering, using autolog ous myoblasts, offer a solution to this problem for female and male patients. Unfortunately, patients in need of engineered tissues and organs are older and therefore exposed or at risk of cancers. Only little is known on the influence of adult stem cells on preexisting tumors. In Urology, MPC cell injection has been used in preclinical studies for the treatment of SUI, a frequent complication of the Radical Prostatectomy, treatment of choice for localized prostate cancer. These cells are injected into the pelvic floor, the most common place of residual tumor cells after surgical treatment. We propose to investigate the safety of stem cell therapy and the interactions between MPCs and preexisting cancer.

Hombauer, H.; Minguell, J. J. (2000) Selective interactions between epithelial tumour cells and bone marrow mesenchymal stem cells. British Journal of Cancer, 82, 1290-1296.

Strasser, H.; Tiefenthaler, M.; Steinlechner, M.; Eder, I.; Bartsch, G., Konwalinka, G. (2000) Age dependent apoptosis and loss of rhabdosphincter cells. The Journal of Urology , Volume 164 , Issue 5 , Pages 1781 - 1785 H.

Mitterberger, M. et al. (2008) Myoblast and fibroblast therapy for post-prostatectomy urinary incontinence: 1-year followup of 63 patients. The Journal of Urology, Volume 179, Issue 1, Pages 226 – 231.

116

Enhanced expression of URI in hepatocellular carcinoma sustains canonical MAP-Kinase signaling and tumor cell proliferation via decreased feedback inhibition of PP1g

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URI is a member of the prefoldin family of chaperones that functions, at least in part, at mitochondria to regulate the mitochondrial threshold for apoptosis in response to nutrient and growth factor availability. The anti-apoptotic role for URI is mediated through its ability to bind to and inhibit PP1g, a known activator of the pro-apoptotic molecule BAD.
Here, we report a new role of URI in tumor cell proliferation in the context of human hepatocellular carcinoma (HCC). HCCs display frequently high levels of URI when compared to non-affected liver tissue. This over-expression is the result of enhanced gene transcription and frequently observed in the context of viral, in particular HBV-driven oncogenesis. In line, the hepatitis B viral protein X, known to be involved in cellular transformation, is a potent transcriptional inducer of URI in HBV-infected Hep3B cells. Over-expression of URI in various HCC cell lines increases cell number, at least in part, due to enhanced cellular proliferation. This effect is dependent on the ability of URI to bind to and inhibit PP1g, suggesting that the URI-PP1g interaction controls beside apoptotic threshold also tumor cell proliferation. On cellular signal transduction level, we identify the pro-proliferative canonical MAP-kinase pathway being controlled by URI/PP1g as well. In accordance, URI over-expression correlates in human HCC with increased MAP-kinase activity, enhanced tumor cell proliferation and ultimately with impaired patient survival.
Our data suggest that human HCCs utilize high levels of URI to lower the feedback inhibition of PP1g on MAP-kinase signaling, leading to enhanced tumor cell proliferation and poor patient outcome.

117

Cancer testis antigen expression and immune responses by prostate cancer patients: implications for prognosis and immunotherapy

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Background. Prostate cancer (PC) is the most frequent malignancy in men and it continues to be one of the most common fatal cancers. Treatment options in advanced castration-resistant prostate cancer (CRPC) are limited. Cancer testis (CT) antigens are expressed in a variety of human cancers, but not in normal tissues except for MHC deficient spermatogonia, and represent promising targets for immunotherapy. Little is known about CT antigen expression in relation to disease progression. The aim of this study was to investigate which CT antigens are expressed and immunogenic and hence represent promising targets for patients with prostate cancer and correlate these findings with clinicopathological characteristics.
Methods. To determine the expression of 6 CT antigens in prostate cancer immunohistochemistry was performed on tissue micro arrays. We investigated 6 CT antigens (NY-ESO.1, MAGE-C1, MAGE-C2, GAGE, MAGE-A1 and MAGE-A4) in benign hyperplasia (n=45), early (n=388) and late stage (n=71) prostate cancer. To determine the occurrence of spontaneous antibodies against cancer testis antigens, ELISA and Western blot was performed for NY-ESO-1, MAGE-C1 and MAGE-C2 with sera from prostate cancer patients.
Results. CT antigens are increasingly expressed in late stage prostate cancers. As an exception we found MAGE-C2 to be expressed early in the course of disease, frequently inducing MAGE-C2 specific antibodies. In later stage metastatic prostate cancer patients NY-ESO-1 is more often expressed, inducing NY-ESO-1 specific antibodies.
Conclusions. Cancer testis (CT) antigens are prognostic markers, frequently inducing immune responses and may be suitable for immunotherapeutic intervention in patients with prostate cancer.

118

Tumor location defines the efficiency of the anti-tumor response

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Glioma are among the most fatal tumors. This has been attributed to immunosuppressive features of both the tumor and the CNS. However, the relative contribution of either the glioma or its localization has not been investigated. We report here that the syngeneic GL261 glioma triggers a protective immune response only when growing subcutaneously, despite the fact that also intracerebrally grown gliomas are infiltrated by DC and T cells. This failure to control intracerebral gliomas correlates with increased immunosuppressive conditions in intracerebral tumors: tumor infiltrating dendritic cells from intracerebral gliomas are not able to stimulate T cell proliferation in vitro; brain-localized GL261 gliomas are characterized by significantly higher numbers of Foxp3+ regulatory T cells and higher expression of TGF-b1 and TGF-b2 mRNA when compared to GL261 gliomas in the skin. Moreover, we show that DCs from intracranial tumors induce in vitro higher numbers of regulatory T cells than subcutaneous tumor DCs. Thus, our data show that not the tumor but its location dictates the efficiency of the anti-tumor immune response.

119

O6-methylguanine DNA methyltransferase (MGMT) promoter methylation in primitive neuroectodermal brain tumor (PNET) correlates with MGMT RNA expression and sensitivity of PNET cells to temozolomide

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Purpose. Methylation of the DNA-repair gene O6-methylguanine-DNA methyltransferase (MGMT) promoter causes gene silencing. This epigenetic modification has been associated with a favorable prognosis in adult patients with glioblastoma who receive temozolomide or other alkylating chemotherapeutic agents. We explored MGMT promoter methylation and expression in pediatric primitive neuroectodermal tumors and cell lines (PNET) and investigated the effect of MGMT methylation/expression on temozolomide (TMZ) and lomustine (CCNU) sensitivity in 7 human PNET cell lines.

Experimental Design. MGMT mRNA and protein expressions of PNET cell lines were measured using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and western blot analysis. The MGMT methylation status of PNET cell lines was determined using a methylation-specific polymerase chain reaction assay and by a more quantitative pyrosequencing assay. MGMT promoter methylation and RNA expression has been assessed in 67 PNET by pyrosequencing assay and by RT-PCR, respectively. We used the MTS assay to assess the sensitivity of PNET cell lines to TMZ and CCNU in relation to MGMT RNA expression.

Results. Med-1 and PNET-5 had higher MGMT mRNA expression compared to normal human cerebellum; D425 was characterized by absence of MGMT expression and MGMT promoter methylation. MGMT methylation was low in most human PNET cell lines and primary PNET and correlated with MGMT RNA expression. Cell viability and MGMT mRNA expression correlated well after TMZ exposure ($p = 0.037$, $r2 = 0.61$), not significantly after CCNU treatment.

Conclusion. In contrast to others tumors - including adult glioblastoma - MGMT appears to be less often methylated in childhood PNET. It remains to be shown whether MGMT methylation status/expression measurement might help identifying patients responding to TMZ and/or other alkylating agents.

Charles Rodolphe Brupbacher Stiftung

Charles Rodolphe Brupbacher Foundation

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.

Portrait by Peter Cerutti

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 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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Index

A

Abdou M 97
Ablukak R 90
Abraham S 71
Aebersold R 53, 97, 102, 107
Aguzzi A 54, 118, 120
Almouzni G 105
Alon U 95
Alt K 57
Amstutz R 81
Anghel S 71
Antsiferova M 59
Arand M 29
Arbogast S 99
Arcaro A 52, 62, 63, 65, 66, 96
Arlt M 78
Arnold I 23
Arnold M 23
Atanackovic D 90
Aubert M 62

B

Bachmann M 99
Ballmer-Hofer K 53
Balmas-Bourloud K 104
Banke I 78
Barbacid M 26
Bar J 31
Barrett M 98
Bartels K 90
Barysch M 58, 69
Basu S 82
Baudis M 81
Baum O 51
Bausch-Fluck D 98, 107
Becher B 109
Beckmann J 67
Beerle C 95
Behnke S 69, 107
Ben-Shabat S 92
Berger C 65, 67
Bergsteinsdottir S 57
Berkovich L 92
Bernasconi L 111
Bernasconi M 67, 84
Bertz J 78
Bianco M 101
Biollaz G 111
Blajecka K 62
Blum H 54
Bock T 107
Bodenmiller B 53
Bode P 100
Bohnert A 79
Bokemeyer C 90
Boller D 63, 65
Bonapace L 78
Bonde A 73
Boneberg E 72
Bornhauser B 78, 83
Born W 78, 105
Bourquin J 67, 78, 83
Boysen G 56
Brand C 58
Brandt S 67
Breitenstein S 74
Bremer J 54
Breuhahn K 87
Brisken C 55
Brümmendorf T 90
Brupbacher F 9, 10, 29, 114, 117, 120
Bubendorf L 98
Buffoli F 101
Bühler P 57
Burg G 91

C

Casagrande S 56
Castelletti D 66
Cattaneo E 91, 101
Cavenee W 28, 33, 120
Cerciello F 98
Chaudhuri A 109
Checchini M 93
Christofori G 86
Cima I 82, 97
Clarke M 29, 49, 120
Claus C 74, 103
Clavien P 28, 54, 74, 120
Cohen A 54, 95
Cohen-Saidon S 95
Cohrs S 53
Colombetti S 71
Compton C 29, 43, 120
Cornils H 59
Correa R 71
Costanzo V 109
Coulon A 67
Cozzio A 58
Cretton C 111
Croce C 29, 39, 120
Curioni-Fontecedro A 108

D

D'Alessandro V 84
Dannenmann S 100
Dedes K 88
Degos L 25
Delattre O 62
de Laurentiis A 62
de Maddalena V 117, 120
Dettmer M 94
Deyev S 92
Dietel M 80
Dip R 104
Dirnhofer S 59
Djonov V 51
Djouder N 102, 110
Doepfner K 52
Dorn D 85
Dorner M 65, 67
Downward J 52
Druker B 26
Dummer R 57, 58, 69, 91, 95, 108
Dymalla S 84
Dziunycz P 63

E

Eberli D 110
Eggimann G 73
Eichhoff O 57, 69
Einarsson S 57
Eisenring M 109
Elsässer-Beile U 57
Elsing A 96
Erlic Z 77

F

Faller G 64
Faoro D 112
Fares F 54
Fattet S 62, 96
Fedida-Metula S 68, 90
Fedier A 88, 89
Feldman Y 90
Felley-Bosco E 70, 74, 75, 87, 98
Fiaschetti G 66
Fink D 88, 89
Fischer A 9, 10, 120
Fischer E 53
Fishman D 55, 68, 90
Flahaut M 67, 104
Follonier C 108
Fontana A 111
Frei C 70
Frei K 63, 111
French L 24, 63, 91, 115
Frew I 103
Friedli A 53
Fritzsche F 60, 74, 75, 79, 80
Fuchs B 49, 72, 78, 105
Fulda S , 29, 46, 120
Furmanova P 76
Fürstenberger G 72

G

Gasser S 28, 28, 120
Geddert H 64
Gehring H 106
Gerhardt J 79
Germann M 93
Gerrits B 107
Gluderer S 93
Grabowska D 96
Grafi-Cohen 54
Graf A 87
Graf R 54
Grässer F 96
Grätz K 9, 10, 117, 118
Greber U 92
Gross N 67, 104
Grotzer M 62, 65, 66, 96, 112
Gruber G 51
Grünberg J 53
Gstaiger M 102
Guerreiro A 62, 96
Gutbrodt K 103

H

Hafen E 93
Hafner J 63
Hajdin K 84
Haldemann A 94
Harnack U 85
Harris C 28, 39, 120
Hashimoto Y 109
Haybaeck J 54, 112
Hecker R 81
Hefermeh L 100
Hegi M 112
Heikenwälder M 54, 77
Hellerbrand C 74, 110
Hemmings 59
Hemmi S 69, 92
Hermanns T 60, 80, 100, 111
Hess D 102
Hildebrandt Y 90
Hitzler I 88
Hlushchuk R 51
Hoeben R 92
Hoek K 57, 69
Hofbauer G 63, 91
Hofmann A 107
Hohl D 49, 58, 59
Höland K 63
Hollenstein A 70
Hoppe-Seyler F 66, 84
Hoppe-Seyler K 66, 84
Hopt U 64
Huber A 53
Huber M 59
Hübscher U 29, 46, 47, 64, 76, 118, 120
Huelsken J 29, 49, 121
Hunger R 58
Hurwitz A 93
Husmann K 105
Hussain S 39
Huynh-Do U 79
Hynx D 59

I

Iezzi G 54
Imesch P 88, 89
Imig J 96
Ingold B 82

J

Jaeger E 111
Jaenisch R 26
Jäger C 79
Jäger P 99
Jaunin F 71
Jiricny J 29, 83, 89, 91, 101, 105, 121
Jochum W 74, 110
Johannsen M 75
Joseph J 67, 91, 121
Jung K 75, 79
Jung M 100

K

Kaaks R , 28, 40, 121
Kaden S 89

Kaegi S 82
Kaelin W 29, 44, 121
Kallioniemi O 29, 42, 121
Karin M 29, 45, 54, 121
Keller L 111
Kleihues P 9, 10, 19, 29, 117
Klein G 25
Klimstra D 64
Knobel P 87
Knudson A 25
Knuth A 29, 69, 72, 77, 108, 118
Kobold S 90
Kohler E 88
Kokowski K 85
Kolm I 58
Komminoth P 94
Kopf M 54
Kosmidis M 63
Kratz K 83
Krek W 82, 97, 102, 103, 110
Kreyden O 58
Kristiansen G 60, 69, 74, 75, 79, 80, 99, 100
Kristiansen I 75
Kröger N 90
Kumar N 90
Kumar S 69, 73
Kumar N 81
Kurrer M 54, 91
Kurtz S 87

L

Laczko E 101
Laghi L 101
Lai C 52
Lane D 25
Lassmann S 64
Läuchli S 63
Legler D 72
Lein M 100
Lenoir G 118, 121
Lerner A 54
Leuba F 71
Levine A 25
Levy F 71
Liewen H 74
Linnebank M 51, 60
Loewith R 53
Lohrey C 84
Lopes M 107, 108, 109
Lütkens T 90
Luu V 55, 56

M

Mäcke H 86
Malz M 87
Marinov M 65
Marra G 91, 101
Martinet D 67
Marti T 87
Mathe E 39
Matter M 103
Meier R 67
Meili A 94
Meister G 96
Melroe G 65
Menigatti M 91
Meraldi P 103
Mete S 73
Metzler S 102, 110
Mihic-Probst D 85, 108
Millard A 76
Miller K 100
Mirkowska P 83
Mittelbronn M 94, 112
Mnich C 95
Moch H 28, 29, 55, 56, 60, 74, 75, 79, 80, 82, 85, 94, 96, 99, 100, 103, 107, 108, 110, 111, 118, 121
Mollenkopf H 100
Montani M 60
Moosleithner-Batliner A 117, 121
Moosmeier M 66
Mori T 108
Morra L 55
Moskau S 60
Moskovits N 31, 32
Mousses S 98
Mueller A 71, 88, 101
Muff R 105
Mühleisen B 63, 91
Mühlethaler-Mottet A 67, 104
Müllhaupt B 74
Muñoz N 6, 8, 9, 10, 11, 12, 15, 28, 37, 121

N

Nachshon-Kedmi M 54
Nadal D 65, 67
Nagamine Y 52
Napso T 54
Nardou K 67, 104
Neelsen K 107
Neubauer J 64
Neumann H 77
Nevins J 28, 28, 35, 121
Niggli F 67, 81
Nitsch R 118, 121
Novak-Hofer I 53
Nuber N 108

O

Ochsenbein A 103
Oeggerli M 98
Oelrich B 75
Old L 26
Opitz I 70, 75, 85
Opitz O 64
Oren M , 28, 32, 121
Osyntsov L 68

P

Paine C 104
Parasuraman P 76
Parkin D 9, 9, 10, 11, 28, 36
Pavelic V 103
Pecher G 85
Pels H 51
Perren A 94

Pestalozzi B 74
Peters R 77
Peto R 6, 8, 9, 10, 19, 20, 23, 28, 41
Petrov I 91
Piguet V 71
Plass C 28, 34, 122
Praetorius C 57
Probst-Hensch N 74
Provenzano M 80, 100, 111
Pruschy M 51, 70, 72, 76
Püntener U 111

Q

Quivy J 105

R

Racay P 102
Rajewsky K 26, 118, 122
Rawandalt N 61
Rechsteiner M 65
Reineke T 97
Renner C 96
Rentsch C 93
Reubi J 86
Riener M 60, 74, 79, 87, 110
Riesterer O 51
RN S 61, 68, 78
Robeson A 98
Rodriguez S 68, 79
Roth P 94
Rotter V 31, 32
Ruf Hrdlicka S 58
Ruiz C 98
Ruminska J 92

S

Sabates-Bellver J 91, 101
Sais G 80, 111
Salenius C 65
Saller E 109
Salm F 62
Samaras P 74
Sayi A 88
Schäfer A 100
Schäfer B 65, 81, 84
Schaffner R 58
Schafhausen P 90
Schärer L 63, 91
Scheffner M 84
Schepsky A 57
Schetter A 39
Schibli R 53
Schiess R 97
Schirmacher P 87
Schlegel N 57, 69
Schlegel U 51, 60
Schmid B 108
Schmidt A 102, 107
Schmidt B 69
Schmidt-Weber C 63
Schmidt-Wolf I 51
Schmitt A 94
Schmitz M 78, 83
Schneider S 98
Schöpf B 105
Schraml P 55, 56, 82, 96, 100, 103
Schrappe M 78, 83
Schrauder A 83
Schreiber R 26, 118, 122
Schubert O 97
Schubiger P 53
Schwaller B 102
Schwarz K 99
Schwendener R 73, 89
Sciuscio D 112
Segal S 68, 90
Sehr P 84
Seifert H 60
Sekler I 90
Semmler A 51, 60
Sendoel A 89
Senn H 72
Shalaby T 62, 65, 66, 96, 112
Shankpal P 61
Shankpal V 61
Sharma A 72
Shi Y 33, 74
Sidi R 75
Sigal A 95
Simon M 60
Sintov A 92
Sion-Vardy N 68
Slan J 95
Smyth M 26
Soll C 74, 87
Soltermann A 55, 99
Sommer L 29, 48, 122
Spagnoli G 80
Speck R 65, 67
Spertini O 52
Stahel R 70, 74, 75, 87
Stahl M 53
Stalder R 71
Stambolsky P 31
Stanulla M 78, 83
Stasche T 90
Stegert M 59
Steinert H 94
Steingrimsson E 57
Stenner F 74, 111
Stephan C 59, 79, 100
Stiehl D 82
Stocker H 93
Stölting M 110
Struckmann K 56
Sulser T 60, 80, 110
Suter T 111
Suzlovich Z 68
Szabo E 89

T

Tang L 64, 80
Thalmann G 93
Theurillat J 110
Thies S 85
Thimme R 54
Thoma C 82, 103
Tinguely M 67, 96, 97, 107

Tischler V 80
Todkar K 102
Toller I 101
Toso A 103

U

Uhlich F 90
Umbricht G 117
Uotila A 53

V

Valgeirsdottir S 57
van den Broek M 69, 72, 100, 108
van Loon B 47, 64, 76
Vent P 91
von Boehmer L 72, 100, 111
von Büren A 66, 112
von Teichman A 55, 56, 82
Voung V 76

W

Wachtel M 81
Wagner E 26
Wagner I 54,
Wagner U 100
Walter A 69, 70, 75, 78, 85, 105
Wang Z 25
Wanka S 53
Weber A 54, 63, 69, 87
Weder W 70, 99
Weinberg R 25
Weller M 33, 94
Werner M 64
Werner S 59
Wetterauer U 57
Wetterwald A 93
Wicki A 86
Wick W 94
Widmer D 57
Wild C 28, 122
Wild D 86
Wild P 28, 56, 60, 86, 86, 110, 111
Wiman K , 28, 32, 122
Wirbelauer C 102
Wojtalla A 65
Wolf M 51, 54, 57
Wolf P 122
Wollscheid B 82, 107
Wood J 51
Wüest 93
Wu F 106
Wyler S 80

Y

Yalcin O 55
Yoshikiyo N 89

Z

Zanetti K 97
Zaugg T 97
Zauner L 97
Zeller K 54
Zellweger T 98
Zeltner T 28, 31, 122
Zhang L 45, 104
Zhu J 33, 96
Ziegler A 98
Zimmermann D 97
Zipser M 69, 95
Zorzi F 101
Zucol F 67
Zur Hausen H 25