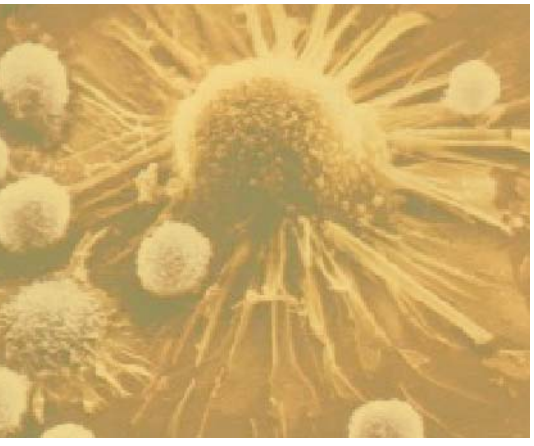
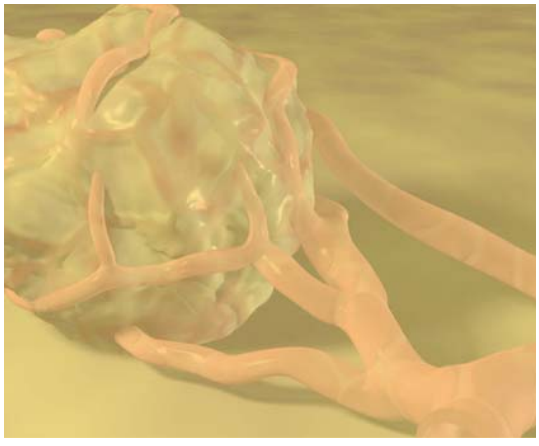
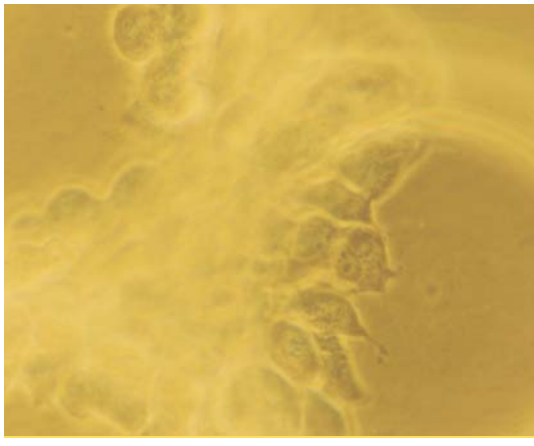
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GTCbio
Global Technology Community

Tumor Progression
& Therapeutic Resistance

October 22-24, 2006 - Baltimore, MD

Sponsored in part by:

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UNIVERSITY OF PENNSYLVANIA
Abramson Cancer Center

Chairperson's Welcome Letter

Dear Colleagues:

This is our 3rd annual multi-disciplinary conference focusing on the problem of drug resistance in cancer. A great deal of knowledge is beginning to unravel the complex molecular and cellular changes associated with malignant tumor progression. With this comes many opportunities for therapeutic development. Featuring the latest tools, models, and research findings, this conference is a great opportunity to learn and develop new approaches and collaborations or contacts. We hope that members of both academia and industry attending the meeting will participate in the discussions.

Our Keynote speaker this year is Dr. Bert Vogelstein, M.D. from Johns Hopkins University. His pioneering studies of the genetic causes of human cancer have made him one of the most cited scientific authorities in the world, and have won him many awards, including the Medal of Honor of the American Cancer Society and the Lounsbery Award of the National Academy of Sciences.

In addition to the scientific sessions, a Roundtable Discussion with NCI Staff will take place to discuss leveraging resources and realigning priorities with limited resources.

So, welcome to Baltimore and enjoy the meeting. I hope you will also get to see some of the many historic and cultural delights the city has to offer.

Please let me know if I can help make this a better meeting for you and please send me any suggestions on things we may wish to do differently.

I would also like to take this opportunity to thank the members of the Organizing Committee for their efforts and many suggestions along the way. The meeting would not have been possible without your input.

Sincerely,
Wafik S. El-Deiry, M.D., Ph.D.
Conference Chairperson

Committee Members

Houston Baker
NCI, NIH

Vimla Band
Northwestern University

Larry Clarke
NCI, NIH

Carlo Croce
Kimmel Cancer Center, Thomas Jefferson University

Bimal Dasmahapatra
Schering-Plough Research Institute

***Wafik El-Deiry**
University of Pennsylvania

Stephen Fesik
Abbott Laboratories

John Glick
University of Pennsylvania

Andrei Gudkov
Cleveland Clinic

Meenhard Herlyn
The Wistar Institute

Mien-Chie Hung
MD Anderson Cancer Center

Russell Kaufman
Wister Institute

Bob Kerbel
Sunnybrook and Women's College Health Sciences Centre

Tak Mak
University of Toronto

Gillies McKenna
University of Pennsylvania

Suresh Mohla
National Cancer Institute

Mark Nelson
Unversity of Arizona

Allen Oliff
GlaxoSmithKline

Martin Padarathsingh
National Cancer Institute

Ramon Parsons
Columbia University

Kornelia Polyak
Dana Farber Cancer Institute

Vivek Rangnekar
University of Kentucky

E. Prem Reddy
Temple University

John Reed
Burnham Institute

Edward Sausville
University of Maryland

Li-Kuo Su
M.D. Anderson Cancer Center

Craig Thompson
University of Pennsylvania

Bert Vogelstein
Howard Hughes Medical Institute

Bin-Bing Zhou
Incyte Corporation

*Conference Chairperson

Schedule At A Glance

NOTE: The general session will take place in the Grand Ballroom for all 3 days. The reception and poster session on Sunday October 22 will take place in the Versailles room on the 1st floor. Breakfast, breaks and lunches will take place in and around the Grand Ballroom.

Sunday, October 22, 2006

2:00 PM **Registration & Poster Set up**

Cancer Genetics, Epigenetics and Genomics I

Session Chair: Scott E. Kern, Johns Hopkins University

3:00 PM **Hypersensitivities versus Reduction-of-fitness: Distinguishing Fact from Artifact in Pharmacogenomic Studies of Cancers having Specific Mutations**
Scott E. Kern, Johns Hopkins University

3:30 PM **Comparative Oncogenomics of Mouse and Human Tumors**
Lynda Chin, DFCI, Harvard University

4:00 PM **New Approaches to the Reactivation of Epigenetically Silenced Genes in Cancer Cells**
William G. Nelson, Johns Hopkins University

4:30 PM **Refreshment Break**

MicroRNAs in Cancer Pathogenesis

Session Chair: George A. Calin, Ohio State University

5:00 PM **Toward a microRNA Revolution in the Cancer Society**
George A. Calin, Ohio State University

5:30 PM **Oncomirs - microRNAs and Cancer**
Frank Slack, Yale University

6:00 PM **Poster Session and Opening Mixer**

Schedule At A Glance *(continued)*

Signaling Pathways and Therapeutic Resistance I

Session Chair: Jeffrey Settleman, MGH, Harvard University

8:00 PM **EGF Receptor Mutants in Human Lung Cancer**
Jeffrey Settleman, MGH, Harvard University

8:25 PM **HOXB7: At the Hub of Multiple Signaling Pathways Leading to Tamoxifen Resistance**
Sara Sukumar, Johns Hopkins University

8:50 PM **Hdm2 and Hdmx: Independent Targets for p53 Activation in Cancer?**
Mark Wade, Salk Institute

9:10 PM **Mitogen-Activated Protein Kinase Phosphatase-1 Signaling in Drug-Resistance**
Gen Sheng Wu, Karmanos Cancer Institute

9:30 PM **End of Day 1**

Monday, October 23, 2006

6:45 AM **Breakfast**

Cancer Genetics, Epigenetics and Genomics II

Session Chair: Spyro Mousses, Tgen

7:30 AM **Application of Novel Cellular Genomics Strategies to Support Cancer Drug Development**
Spyro Mousses, Tgen

7:55 AM **Genetic Lessons from Colon Cancer**
Sandy Markowitz, Case Western Reserve University

8:20 AM **Genomic Analyses of Human Cancer**
Victor Velculescu, Johns Hopkins University

8:45 AM **Refreshment Break**

Schedule At A Glance

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KEYNOTE ADDRESS

9:00 AM **Translational Implications of Colorectal Cancer Genetics**
Bert Vogelstein, M.D., Professor of Oncology & Pathology, Johns Hopkins University, Investigator, Howard Hughes Medical Institute

10:00 AM **Refreshment Break**

Tumor-Stromal Interactions and Tumor Microenvironment

Session Chair: Mary Hendrix, Northwestern University

10:15 AM **Targeting the Plasticity of Metastatic Tumor Cells**
Mary Hendrix, Northwestern University

10:45 AM **Tumor Microenvironment in Cancer Progression and Metastasis**
Raghu Kalluri, BIDMC, Harvard University

11:15 AM **Cellular Senescence and the Tumor Microenvironment**
Judy Campisi, Berkeley National Lab

11:45 AM **Strategies for Modification of Host Stroma for Resistance to Tumor Growth**
Vivek Rangnekar, University of Kentucky

12:10 PM **Special Lunch Session: Panel Discussion on NIH Funding and Survival Opportunities**

Suresh Mohla, Mary Wolpert, Jorge Gomez, Houston Baker, Joanna Watson, Chi Dang, Ben Neel, William Nelson

Cancer Stem Cells

Session Chair: Edward F. Srour, Indiana University School of Medicine

2:00 PM **Defining Subpopulations of Breast Cancer Stem and Progenitor Cells**
Edward F. Srour, Indiana University School of Medicine

Schedule At A Glance *(continued)*

2:25 PM **Disruption of Self-Renewal in Cancer**
Michael F. Clarke, Stanford University

2:55 PM **Molecular Profile of Breast Stem Cells**
Kornelia Polyak, DFCI, Harvard University

3:20 PM **Refreshment Break**

Signaling Pathways and Therapeutic Resistance II

Session Chair: Chi Van Dang, Johns Hopkins University

3:40 PM **Therapeutic Implications of the c-Myc Target Gene Network**
Chi Van Dang, Johns Hopkins University

4:10 PM **Fatty Acid Synthase: A Metabolic Oncogene in Breast Cancer**
Ruth Lupu, Northwestern University

4:35 PM **Shuttling of Membrane Receptors and Cytoplasmic Molecules to the Nucleus: Importance to Tumor Progression**
Mien-Chie Hung, Univ. of Texas MD Anderson Cancer Center

5:00 PM **From Somatic Genetic Alterations in Cancer to Therapeutic Intervention**
William Sellers, Novartis Institutes for Biomedical Research

5:30 PM **POSTER AWARDS**

6:30 PM **Dinner**

Models of Human Cancer Progression I

Session Chair: Gerard Evan, UC San Francisco

7:30 PM **Uses and Abuses of Apoptosis in Cancer**
Gerard Evan, UC San Francisco

8:00 PM **TGF-beta/Smad Signaling in Pancreatic Cancer Progression**
Nabeel Bardeesy, MGH Cancer Center, Harvard University

8:30 PM **Mouse Models of Breast Cancer Progression and Maintenance**
Lewis Chodosh, University of Pennsylvania

Schedule At A Glance

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Tuesday, October 24, 2006

7:00 AM **Breakfast**

Mechanism-Driven Therapeutics I

Session Chair: Richard F. Wooster, GlaxoSmithKline

7:30 AM **The Role of Biomarkers that Predict Response to Targeted Therapeutics**
Richard F. Wooster, GlaxoSmithKline

8:00 AM **Whole Genome Evolution Technology: Development of Therapeutic Antibodies Specific for Cancer Pathways**
Nicholas Nicolaides, Morphotek Inc.

8:25 AM **Targeting the Cell Cycle Machinery for Cancer Therapy**
E. Prem Reddy, Fels Institute

8:55 AM **Magic Combinations of Anticancer Drugs**
Mikhail Blagosklonny, Ordway Institute

9:20 AM **Refreshment Break**

9:30 AM **Oral Presentations from Selected Abstracts**

10:40 AM **Refreshment Break**

Models of Human Cancer Progression II

Session Chair: Ramon E. Parsons, Columbia University

10:50 AM **Pathogenesis of Breast Cancer: the PTEN and PI3-K Pathway**
Ramon E. Parsons, Columbia University

11:15 AM **PTEN Phenotypes in Human Cancer Cells**
Todd Waldman, Georgetown University

11:40 AM **The Shp2/Ras/Erk Pathway in Human Genetic Disease and Cancer**
Ben Neel, BIDMC, Harvard University

Schedule At A Glance *(continued)*

12:05 PM **Lunch**

Molecular Imaging of Cancer

Session Chair: Wafik El-Deiry, University of Pennsylvania

1:00 PM **Imaging Approaches for Cancer Drug Development**
Wafik El-Deiry, University of Pennsylvania

1:25 PM **Translational Molecular Imaging for Oncology**
Martin G. Pomper, Johns Hopkins University

1:50 PM **Use of Imaging to Optimize Cell-Based Therapies for Cancer**
Christopher H. Contag, Stanford University

2:15 PM **Refreshment Break**

Bioinformatics and Cancer Proteomics

Session Chair: Samir Hanash, Fred Hutchinson Cancer Research Center

2:30 PM **Proteomics Approaches to Tumor Antigen Identification**
Samir Hanash, Fred Hutchinson Cancer Research Center

2:55 PM **Bioinformatics as an Engine for Oncology Discovery**
Arul Chinnaiyan, University of Michigan

3:20 PM **Refreshment Break**

Mechanism-Driven Therapeutics II

Session Chair: Timothy MacLachlan, Genzyme Corporation

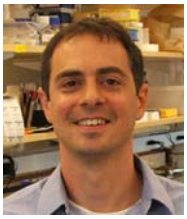
3:30 PM **TGF-beta Neutralizing Antibody GC10 AM0 AM8 for Melanoma and Renal Cancer**
Timothy MacLachlan, Genzyme Corporation

3:55 PM **Exploiting Endoplasmic Reticular Stress in Cancer Therapy**
David McConkey, MD Anderson Cancer Center

4:20 PM **Telomere Targeting Agents**
Angelika Burger, University of Maryland

4:45 PM **Conference Concludes**

Invited Abstracts



Nabeel Bardeesy
MGH, Harvard University

Nabeel Bardeesy in an Assistant Professor of Medicine at Massachusetts General Hospital and Harvard Medical School. He obtained his Ph.D. from McGill University where he studied the molecular genetics of Wilms' tumor (nephroblastoma). His postdoctoral training was conducted with Dr. Ron DePinho at the Dana-Farber Cancer Institute where he focused on applying conditional knockout approaches to generate accurate mouse models of human cancers. This work provided a detailed view of the tumor biological effects of a series of important cancer genes, including a) confirming the tumor suppressor activity of Lkb1 in vivo, b) demonstrating the requirement for lesions in both the Rb and Ink4a pathways in melanoma development and c) demonstrating critical roles for Kras activation in regulating pancreatic adenocarcinoma initiation and of Ink4a/Arf in cancer progression. These latter studies established a faithful mouse model of pancreatic adenocarcinoma. His laboratory, initiated in 2005, continues to focus on studies of pancreatic cancer biology. He has now generated a series of mouse models that interrogate all of the signature mutations of the human disease. Specific interests include the investigation of the link between developmental signaling pathways and cancer initiation, and the use of genetically engineered mouse models in cancer gene discovery.

TGF-beta/Smad Signaling in Pancreatic Cancer Progression

The *SMAD4* tumor suppressor gene is inactivated in the majority of pancreatic ductal adenocarcinomas (PDAC) with concurrent mutational inactivation of the *INK4A/ARF* tumor suppressor locus and activation of the *KRAS* oncogene. Here, using genetically engineered mice, we determined the impact of *SMAD4* deficiency on the development of the pancreas and on the initiation and/or progression of PDAC – alone or in combination with PDAC-relevant mutations. Selective *SMAD4* deletion in the pancreatic epithelium had no discernable impact on pancreatic development or physiology. However, when combined with the activated $KRAS^{G12D}$ allele, *SMAD4* deficiency enabled rapid progression of $KRAS^{G12D}$–initiated neoplasms. While $KRAS^{G12D}$ alone elicited pre-malignant pancreatic intraepithelial neoplasia (PanIN) that progressed slowly to carcinoma, the combination of $KRAS^{G12D}$ and *SMAD4* deficiency resulted in the rapid development of tumors resembling intraductal papillary mucinous neoplasia (IPMN), a precursor to PDAC in humans. *SMAD4* deficiency also accelerated PDAC development of $KRAS^{G12D}$ *INK4A/ARF* heterozygous mice and altered the tumor phenotype; while tumors with intact *SMAD4* frequently exhibited epithelial-to-mesenchymal transition (EMT), PDAC null for *SMAD4* retained a differentiated histopathology with increased expression of epithelial markers. *SMAD4* status in PDAC cell lines was associated with differential responses to TGF-β *in vitro* with a subset of SMAD4 wildtype lines showing prominent TGF-β induced proliferation and migration. These results provide genetic confirmation that *SMAD4* is a PDAC tumor suppressor, functioning to block the progression of $KRAS^{G12D}$-initiated neoplasms, whereas in a subset of advanced tumors, intact *SMAD4* facilitates EMT and TGF-β-dependent growth.



Mikhail V. Blagosklonny
Ordway Institute

Mikhail V. Blagosklonny received an M.D. and Ph.D. from Pavlov Leningrad Medical University (St-Petersburg, Russia) and then conducted pre-clinical and clinical investigations. In 1991, he moved to the National Cancer Institute (Bethesda, MD) to study malignant transformation and experimental anticancer drugs. While with the NIH, he also was involved in clinical trials of tamoxifen in prostate cancer patients. Currently, he is a senior scientist at the Ordway Research Institute, Albany, NY. He is an author of 150 publications and a member of Editorial Boards of Cancer Res, Cell Death Differ, Cancer Biol Ther, PLOS One, Int J Cancer and Cell Cycle. Dr. Blagosklonny's research interests range from molecular and cellular biology to clinical investigations and specifically include oncogenes and tumor suppressors, signal transduction, cell cycle, mitosis, apoptosis, anticancer therapeutics with emphasis on translation of basic science into new anticancer strategies such as exploiting cancer cell cycling and drug resistance for selective protection of normal cells. Certain scientific problems can be solved by linking seemingly unrelated phenomena. Therefore, to complement his own experiments, he retrieves and re-analyzes published data often from seemingly unrelated fields, thus generating new conclusions using pre-existing experiments. Currently, he has extended this approach from cancer to other age-related diseases and aging itself, as a result suggesting a potentially anti-aging drug to be used today (Cell Cycle, 2006, 5 (18): 2087 - 2102).

Magic Combinations of Anticancer Drugs

A magic bullet is a hypothetical drug that eradicates cancer but does not affect normal cells (like penicillin for bacterial infections). The closest example is Gleevec for CML, although it is still far from being selective and effective. Potent drugs inhibit universally vital targets (microtubules, DNA, topoisomerases, proteasomes, HDAC), thus causing side-effects that limit therapy of cancer. A few inhibitors of relatively cancer-specific pathways either are not effective or resistance develops. Although better targets will be identified, a magic bullet is unlikely, at least in the near future. Yet, what cannot be achieved by using one drug can be achieved by combining selective and cytotoxic drugs aimed at matching targets. Such magic combinations can preferentially kill cells with defective G1 and G2 checkpoint, restriction points, expressing drug pumps or lacking tumor suppressors. Magic combinations can be antagonistic in cells with normal characteristics, while synergistic in cancer cells. A complementary principle is targeting cells with two (or more) characteristics that are normally mutually exclusive, such as rapid cycling plus mitogen-independence, cycling plus high expression of drug transporters, or cycling plus stem cell markers, for example, attacking proliferating cancer stem-like cells (stemloids), while sparing normal (quiescent) stem cells. Unlike conventional combinations, magic combinations can only be validated in paired cell lines, by killing one cell type without killing another. Given a great number of possible combinations, the empirical approach is impractical. Based on cell cycle and signal transduction

pathways, it is possible to rationally design a combination (therapeutic engineering) to target any characteristic, including drug resistance. The success of this approach will depend on the development of companion molecular diagnostics. Finally, I discuss a business model for development of magic combinations as multi-pills and kits as well as development of drug analogs with improved characteristics, precisely adjusted for magic combinations.



Angelika Burger
University of Maryland

Dr. Burger is an associate professor of pharmacology and experimental therapeutics at the University of Maryland, School of Medicine and a researcher within the Experimental Therapeutics Program of the University of Maryland Marlene and Stewart Greenebaum Cancer Center. She received Ph.Ds in pharmaceutical chemistry from the Johannes-Gutenberg-University in Mainz, Germany, and in cancer research from the University of Bradford, Bradford, England. Dr. Burger held postdoctoral training positions at the National Cancer Institute, Developmental Therapeutics Program, and at the Tom Connors Cancer Center, University of Bradford, England.
Her research focus is the identification and validation of new molecular targets and molecularly targeted agents. Her particular interest is in the development of telomerase inhibitors for cancer therapy and defining their potential as cancer stem cell-directed treatments. She has been involved in defining a new class of telomerase inhibitory agents, termed telomere targeting agents, for which she was recognized with the Science Prize of the German Cancer Society 2005. Dr. Burger is the author of over 70 original articles and editorial board member of Clinical Cancer Research, European Journal of Cancer and British Journal of Cancer. She is also the chairman of the European Organization for Research and Treatment of Cancer Drug Discovery Committee.

Telomere Targeting Agents

Telomeres are repetitive sequences of duplex DNA (TTAGGG) with a 3'-prime single stranded overhang that can fold into a 4-stranded guanine quadruplex structure (G4). The latter has recently been resolved by x-ray crystallography. The telomeric sequences and their associated proteins cap the ends of chromosomes. Telomere maintenance at an adequate length and/or a capped state represents an essential property of all malignant tumors and is achieved by reactivation of the ribonucleo-protein enzyme telomerase. Telomerase activation has been shown to play a critical and early role during the immortalization and stepwise transformation of normal into tumor cells.
Hence, strategies aimed to target the telomere/telomerase complex may lead to a new class of broad-spectrum, specific anticancer drugs. By using a structure-based, rational approach, G-quadruplex ligands were designed to induce the 3' single-stranded guanosine-rich telomeric overhang to fold into a G-quadruplex structure. Formation of a quadruplex DNA structure is incompatible with telomerase attachment to the 3'-end, suggesting that quadruplex-binding and quadruplex-promoting small molecules can in principle effectively inhibit both, the catalytic and capping functions of telomerase. The lead agents are a 3,6,9 trisubstituted acridine, termed BRACO19, and a pentacyclic acridine

named RHPS4. We have shown that these quadruplex-binding ligands, which are potent in terms of telomerase inhibition and quadruplex affinity, can also produce telomere shortening and replicative senescence in cancer cells. *In vitro* studies in cell lines with short and long telomeres demonstrated a rapid induction of telomere-associated DNA damage signaling and the occurrence of mitotic abnormalities in tumor cells with short telomeres. Measurements of pharmacodynamic effects of tumor response in human tumor xenograft models *in vivo* were consistent with the *in vitro* results. For example BRACO19 had single agent activity in an early stage xenograft model with short telomeres. *In vivo* efficacy was associated with loss of expression of the telomerase catalytic subunit and the occurrence of atypical mitoses in tumor tissues, indicating mitotic catastrophe and telomere dysfunction. These effects were dramatically enhanced when either BRACO19 of RHPS4 were combined with taxol.

Owing to the direct interaction of BRACO19 and RHPS4 analogs with telomeres and the only indirect inhibition of telomerase, we named these drugs telomere targeting agents (TTA).

I will give an overview of avenues for targeting telomeres and telomerase and the usefulness of TTAs as anticancer agents.



George A. Calin
Ohio State University

George Adrian Calin received both his M.D. and Ph.D. degrees at Carol Davila University of Medicine in Bucharest, Romania. After completing a cancer genomics training in Dr. Massimo Negrini's laboratory at University of Ferrara, Italy, he became a postdoctoral fellow at Kimmel Cancer Center in Philadelphia, PA, in Dr. Carlo Croce's laboratory. He is presently an Assistant Professor at Ohio State University and studies in his laboratory are focused on the roles of microRNAs and other non-coding RNAs in cancer initiation and progression as well as the mechanisms of cancer predisposition.

Toward a microRNA Revolution in the Cancer Society

Alterations in miRNA genes play a critical role in the pathophysiology of many, perhaps all, human cancer: cancer initiation and progression can involve microRNAs (miRNAs) - small non-coding RNAs that can regulate gene expression. At the present time, the main mechanism of microRNoma (defined as the full complement of microRNAs present in a genome) alteration in cancer cells seems to be represented by aberrant gene expression, characterized by abnormal levels of expression for mature and/or precursor miRNA sequences in comparison with the corresponding normal tissues. Loss or amplification of miRNA genes has been reported in a variety of cancers and altered patterns of miRNA expression may affect cell cycle and survival programs. Germline and somatic mutations in miRNAs or polymorphisms in the mRNAs targeted by miRNAs may also contribute to cancer predisposition and progression.

The causes of the widespread differential expression of miRNA genes between malignant and normal cells can be explained by the genomic location of these genes in cancer-associated genomic regions, by epigenetic mechanisms as well as by alterations of

members of the processing machinery. MicroRNAs expression profiling has been exploited to identify miRNAs that are potentially involved in the pathogenesis of human cancers. MicroRNAs profiling achieved by various methods has allowed the identification of signatures associated with diagnosis, staging, progression, prognosis and response to treatment of human tumors.



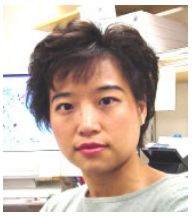
Judith Campisi
Berkeley National Lab.

Judith Campisi received a Ph.D. from the State University of New York and postdoctoral training at the Dana Farber Cancer Institute. She was Assistant and Associate Professor in Biochemistry at the Boston University Medical School, and joined the Lawrence Berkeley National Laboratory as a Senior Scientist in 1991. In 2002, she moved part of her laboratory to the then newly-founded Buck Institute for Age Research. Campisi's work bridges the fields of cancer and aging, and includes contributions to understanding the evolution and mechanisms of tumor suppression, the cellular damage responses of senescence and apoptosis, DNA repair mechanisms, telomere biology, and the role of genome maintenance systems in postponing aging and cancer. She has published >150 scholarly papers on her work, and has received several awards for her research. Her awards include two MERIT awards from the National Institute on Aging, a Senior Scholar Award from the Ellison Medical Foundation, the Irving Wright Award from the American Federation for Aging Research and the Glenn Foundation Award from the Gerontological Society of America. She serves or served on several public scientific advisory panels, including the National Advisory Council on Aging, the American Association for Cancer Research's Task Force on Aging and Cancer and the Alliance for Aging Research's Scientific Advisory Board. She currently serves on the editorial boards of numerous scholarly journals.

Cellular Senescence and the Tumor Microenvironment

Cellular senescence is a tumor suppressive response that irreversibly arrests the proliferation of cells that are damaged or otherwise at risk for transformation. The senescence response may be antagonistically pleiotropic, protecting organisms from cancer early in life, but contributing to aging and age-related disease late in life. This antagonistic pleiotropy arises from the fact that the senescence growth arrest is accompanied by wide ranging changes in gene expression. These changes include the upregulation of genes encoding a variety of secreted growth factors, matrix remodeling enzymes and inflammatory cytokines, a senescence-associated attribute we term the senescent secretory phenotype. The senescent secretory phenotype can disrupt the structure and function of normal tissues. Ironically, it can also promote the proliferation, invasive migration and malignant transformation of nearby premalignant cells. Thus, as predicted by antagonistic pleiotropy, the senescence response protects organisms from cancer by a cell-autonomous suppression of cell proliferation; however, it can promote the development of cancer by the cell-nonautonomous effects of the senescent secretory phenotype, which creates a tissue microenvironment that promotes cancer progression. Several lines of evidence indicate that the p53 tumor suppressor protein is crucial for both

establishing and maintaining the senescence growth arrest. p53 collaborates with the p16 tumor suppressor protein, which renders the growth arrest irreversible. In contrast to the senescent growth arrest, p53 does not positively regulate the senescent secretory phenotype. Rather, p53 actively restrains it. Inactivation of p53 in undamaged presenescent cells did not activate the secretory phenotype, but subsequent irradiation induced a strong secretory phenotype, exceeding that of control cells (damaged cells with wild-type p53). Moreover, inactivation of p53 in senescent cells that express little or no p16 reversed the senescent growth arrest, but the resulting proliferating cells were genomically unstable and had an exaggerated secretory phenotype. Our results indicate that the senescence growth arrest can be uncoupled from the secretory phenotype, and suggest that loss of p53 function puts cells at risk for developing a phenotype that can fuel the development of cancer by influencing the tumor microenvironment.



Lynda Chin
DFCI, Harvard University

Dr. Lynda Chin is a graduate of Brown University and the Albert Einstein College of Medicine and conducted her clinical and scientific training at Columbia Presbyterian Medical Center and the Albert Einstein College of Medicine where she served as Chief Resident of Dermatology. Dr. Chin was recruited to Dana-Farber Cancer Institute and Harvard Medical School in 1998 and is currently an associate professor of dermatology. Dr. Chin has made multiple important scientific discoveries spanning the fields of transcription, mouse models of human cancer, and oncogenomics. Dr. Chin is best known for development of the first mouse model of spontaneous cutaneous melanoma that recapitulates many of the biological and genetic features of the human disease. She has also pioneered the use of conditional transgenic alleles encoding dominantly acting oncogenes for assessment of the role of oncogenes in tumor maintenance. As the Scientific Director of the Belfer Cancer Genomics Center at DFCI, Dr. Chin has led the efforts to develop programmable high-resolution array-CGH technologies on 60mer oligo microarrays. Her study was the first to demonstrate the feasibility of full-complexity genomic DNA hybridization to 60mer oligo microarrays for copy number profiling. Dr. Chin has been at the forefront of applications of high-resolution array-CGH in characterization of human oncogenomes. Her most recent work has focused on comparative oncogenomics of mouse and human cancers for discovery of novel cancer genes.

Comparative Oncogenomics of Mouse and Human Tumors

Extensive molecular and cytogenetic analyses have laid a solid foundation of our understanding of solid tumor pathogenesis but have also underscored the fact that we have only a very limited knowledge of the compendium of genetic lesion(s) driving its genesis, progression and maintenance. In the era of targeted therapy, it is recognized that the identification and elucidation of these genetic lesions represents a first step toward productive drug development effort. We believe that a fully integrated cancer gene discovery program anchored on recurrent genomic changes in human tumors defined by programmable high-resolution array-CGH analyses complemented by refined genetically engineered mouse models

represents a productive approach toward such goals.

Recurrent copy number alterations are clonally selected for by evolving tumor cells thus the targets of these amplification or deletion events represent strong candidates for bona fide cancer genes. Advances in copy number profiling technology have enabled us to categorize these changes with high resolution. For example, in our recent analyses of 88 melanoma samples and 32 melanoma cell lines, we have identified many recurrent focal copy number aberration events with no known or validated targets, representing rich starting point for melanoma gene discovery. On the other hand, embedded within such highly rearranged human cancer genomes are both "drivers" and "passengers" events. Relevant biological filters are necessary to cull non-essential players to prioritize limited resource on functional workup of high-potential driver events. Here, the genetically engineered models serve as a powerful genetic system. We will present examples of how these refined mouse models can help prioritize one's efforts in cancer gene discovery.



Arul M. Chinnaiyan, M.D., Ph.D.
University of Michigan

Dr. Chinnaiyan is the S.P. Hicks Endowed Professor of Pathology and Professor of Pathology and Urology at the University of Michigan Medical School. In addition to receiving his medical training at Michigan, he received his Ph.D. in Pathology and has made seminal contributions to the understanding of the molecular mechanisms of apoptosis (programmed cell death). Dr. Chinnaiyan has received a number of awards including the Basic Science Research Award awarded by the University of Michigan Medical School Dean's Office, the AMGEN Outstanding Investigator Award, the Pew Biomedical Scholar Award, the Burroughs Welcome Foundation Award in Translational Research and the 2006 Benjamin Castleman Award.

Dr. Chinnaiyan is a board certified Clinical Pathologist and leads the Pathology Department's effort in the emerging field of Clinical Genomics. He currently serves as Director of the Division of Pathology Research Informatics and Director of Cancer Bioinformatics. He also established and currently directs the Pathology Microarray Laboratory for the Department of Pathology.

Dr. Chinnaiyan's basic science/translational laboratory has focused on functional genomic, proteomic and bioinformatics approaches to study cancer for the purposes of understanding cancer biology as well as to discover clinical biomarkers. He and his collaborators have characterized a number of biomarkers of prostate cancer including AMACR, EZH2 and hepsin. Most recently, his laboratory identified recurrent gene fusions of TMPRSS2 to ETS family transcription factors in prostate cancer, potentially redefining the molecular basis of prostate cancer as well as other common epithelial cancers. His laboratory developed the popular cancer profiling bioinformatics resource called Oncomine (www.oncomine.org) which is freely available to the academic community (hosting nearly 10,000 registered users from over 30 countries).



Lewis Chodosh
Univ. of Pennsylvania

Dr. Chodosh graduated from Yale University in 1981 with a degree in Molecular Biophysics and Biochemistry. He obtained his M.D. from Harvard Medical School in 1989 along with a Ph.D. in Biochemistry from M.I.T., where he carried out his graduate work in Dr. Phillip Sharp's laboratory on transcription factor biochemistry. After completing his internship and residency training in internal medicine as well as a fellowship in endocrinology at the Massachusetts General Hospital, Dr. Chodosh went on to perform postdoctoral work on breast cancer biology and genetics in Dr. Philip Leder's laboratory at Harvard Medical School.

In 1994, Dr. Chodosh joined the faculty of the University of Pennsylvania where he currently serves as Director of Cancer Genetics for the Abramson Family Cancer Research Institute, Vice Chairman of the Department of Cancer Biology, co-Director of the University of Pennsylvania Small Animal Imaging Facility, and Associate Director of the Abramson Cancer Center for Cancer Biology. The Chodosh laboratory uses genetically engineered mouse models to study the genes and mechanisms that cause breast cancer and that regulate normal mammary gland development. Particular areas of interest include oncogene "addiction", the function of oncogenes and tumor suppressor genes in breast cancer initiation and progression, the biology of tumor dormancy and recurrence, the role of stem cells in breast cancer and mammary development, the mechanisms by which reproductive events alter breast cancer susceptibility, and the use of genomics, computational approaches, and non-invasive imaging to understand mammary development and tumor biology. Dr. Chodosh serves as Director of a Congressionally-Directed Breast Cancer Center of Excellence and is Principal Investigator for one of the sites of the National Cancer Institute's Mouse Models of Human Cancer Consortium. He is a past recipient of the Charles E. Culpeper Foundation Scholar in the Medical Sciences and presently serves as Senior Editor of Breast Cancer Research and an Advisor to the Nurses' Health Study.

Mouse Models of Breast Cancer Progression and Maintenance

The natural history of breast cancer is characterized by the progressive selection and outgrowth of cells that possess increasingly aggressive properties. This process ultimately leads to resistance to therapeutic agents, metastasis, tumor dormancy, and recurrence. Together, these manifestations of tumor progression are responsible for the vast majority of breast cancer deaths. Nevertheless, the mechanisms underlying tumor progression are poorly understood. To better define the molecular, cellular, and physiological events that contribute to breast cancer progression, we have developed a series of inducible transgenic mouse models for this disease that recapitulate critical features of its natural history. These models utilize the tetracycline regulatory system to conditionally express oncogenes relevant to human cancer in the mammary epithelium of transgenic mice. Following induction with doxycycline, transgenic animals develop invasive mammary adenocarcinomas in a manner that is strictly doxycycline-dependent. However, the majority of these tumors are oncogene "addicted" and regress to a clinically undetectable state following doxycycline withdrawal. Nevertheless, despite the dramatic regression of primary tumors that occurs following abrogation of oncogene expression,

many fully regressed tumors recur in the absence of oncogene expression following a latent period and lead to the death of the host. Using these models, we have attempted to identify regulatory molecules that contribute to breast cancer progression of mice and in women.



Michael F. Clarke
Stanford University

Dr. Michael Clarke is a Professor in the Department of Medicine, Division of Oncology Medicine and the Karel H. and Avice N. Beekhuis Professor in Cancer Biology. In addition to his role in the Oncology Division, he is also the Associate Director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine. His expertise in stem cell research resulted in the isolation of cancer stem cells from breast tumors. His work will bolster efforts under way at Stanford to isolate cancer stem cells in different types of tumors. After receiving his MD degree from Indiana University, Dr. Clarke was an Oncology Fellow at the National Cancer Institute. In 1986, he joined the faculty at the University of Michigan where he was a Professor of Internal Medicine and a Professor of Cell and Developmental Biology.
Dr. Clarke was the first scientist to isolate cancer stem cells from a solid tumor. His findings describing the identification of breast cancer stem cells, published in 2003, explained fallibility in current breast cancer treatments and will lead to more effective therapies for numerous types of common malignancies such as ovarian, colon, lung, prostate and head and neck cancers.

Disruption of Self-Renewal in Cancer

Most common cancers 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 cells in many tissues. Solid tumors consist of a heterogeneous population of cancer cells which 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 cancer, a small, 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 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. It is likely that targeting cancer cell self-renewal pathways will result in more effective cancer therapies.



Chi Van Dang
Johns Hopkins University

Chi Van Dang is The Johns Hopkins Family Professor in Oncology Research and is Vice Dean for Research of Johns Hopkins University School of Medicine. He oversees the Hopkins Institute for Cell Engineering and is a Professor of

Medicine, Pathology, Oncology, and Cell Biology with joint appointment in Molecular Biology and Genetics. Born in Saigon, Viet Nam, Dr. Dang immigrated to the USA at age 12 and received his B.S. (highest honors) from the University of Michigan, Ann Arbor. He earned his Ph.D. (distinction) in Chemistry from Georgetown University and M.D. (Alpha Omega Alpha; Phi Beta Kappa) from the Johns Hopkins University School of Medicine in 1982. He was an Osler Medical Resident at the Johns Hopkins Hospital and a Hematology-Oncology fellow at the UCSF Cancer Research Institute. Dr. Dang is Senior Editor of Cancer Research and Associate Editor of Journal of Molecular Medicine. He serves on editorial boards of Current Cancer Therapy Reviews, Drug Discovery Today, Journal of Clinical Investigation, Molecular and Cellular Biology and Neoplasia. He was a member of the NCI Board of Scientific Counselors and was elected president of the American Society for Clinical Investigation (2003).

Therapeutic Implications of the c-Myc Target Gene Network

The MYC oncogene emerges over the past decade at the crossroads of many tumorigenic pathways, whereby MYC activation contributes to at least 30% of US cancer deaths annually. In vitro cell culture models and in vivo xenograft and transgenic MYC tumorigenesis models have strongly implicated the role of MYC in altered tumor metabolism and adaptation to the tumor environment. The network of Myc target genes has been extensively delineated using both oligonucleotide expression arrays and genome-wide chromatin immunoprecipitation studies to identify direct Myc targets in a human inducible neoplastic B cell P493 model. We have previously linked Myc to altered tumor glucose metabolism and shown that LDHA is compelling target for therapy. Now, the spectrum of target genes uncovered in our studies has allowed us to identify novel transcriptional regulatory circuitries and to link Myc and E2F1 to the regulation of nucleotide metabolism and DNA replication. These novel findings are not only of therapeutic interest, but they also provide substantial ground for our understanding of the potential anti-tumorigenic effects of mycophenolic acid (inhibitor of the Myc target IMPDH2) and leflunomide (inhibitor of the Myc target DHODH). Current strategies to target Myc itself will be reviewed.



Wafik El-Deiry
University of Pennsylvania

Wafik S. El-Deiry, M.D. Ph.D. is a Professor of Medicine, Genetics, and Pharmacology at the University of Pennsylvania School of Medicine where he is also a Co-Program Leader of Radiation Biology at the Abramson Comprehensive Cancer Center. Dr. El-Deiry has received many honors and awards and has been recognized by the Institute for Scientific Information as a Highly Cited Researcher. Dr. El-Deiry serves as Editor-in-Chief of the peer-reviewed journal Cancer Biology and Therapy and has been an Active Member of the American Association of Cancer Research's Science Policy and Legislative Affairs Committee. He earned his Bachelor's degree in chemistry in 1981, and M.D. and Ph.D. degrees from the University of Miami in Florida in 1987. He completed his medical residency and fellowship training at the Johns Hopkins Hospital in medicine and oncology prior to joining the faculty at the University of Pennsylvania in 1994. He has published well-over 200 original scientific manuscripts and reviews and has edited

several books on cancer. He serves on numerous national science review panels, is a popular invited speaker, and has trained and mentored several dozen post-doctoral scientists and graduate students. As a practicing physician he specializes in oncology and as a researcher he is looking for new treatments to fight aggressive resistant cancers.



Gerard Evan
UC San Francisco

Gerard Evan received his MA in Biochemistry from the University of Oxford in 1977, and his Ph.D. in Molecular Immunology in 1981 from the University of Cambridge and Medical Research Council (MRC) Laboratory of Molecular Biology. From 1982-84 he was a Post-Doctoral Fellow in the laboratory of J. Michael Bishop at UCSF, returning to the UK to become an Assistant Member of the Cambridge Branch of the Ludwig Institute for Cancer Research. In 1988 he joined the Imperial Cancer Research Fund Laboratories in London where he remained until moving to UCSF in 1999. In 1995, Dr Evan was awarded the Pfizer prize in Biology. In 1996 he awarded the Royal Society's Napier Chair of Cancer Research, elected to the European Molecular Biology Organization and won the Josef Steiner Prize in Cancer Research of the Swiss Oncological Society. He was elected a Fellow of the UK Academy of Medical Sciences in 1999, later that year taking up the Gerson and Barbara Bass Bakar Distinguished Professor of Cancer Biology at UCSF. He was elected to the Royal Society of London in 2005. He currently holds the Neal Levitan Research Chair from the Brain Tumor Society and is a Senior Scholar of the Ellison Medical Research Foundation for Aging.

Uses and Abuses of Apoptosis in Cancer

Cancers arise by evolutionary processes that involve the protracted acquisition by somatic cells of suites of interlocking mutations that uncouple proliferation, survival, migration and damage responses from the selective pressures that normally restrain or restrict them. The relative rareness of cancer cells within the soma, in the face of huge numbers of available cell targets, substantial rates of mutation, and an abundance of proto-oncogenes and tumor suppressor gene targets underscores the restricted evolutionary space available to incipient tumor cells. Such restriction is enforced by intrinsic tumor suppression – the obligatory coupling of growth-promoting programs to growth suppressive processes like apoptosis and senescence. One example of intrinsic tumor suppression is the induction of apoptosis by the Myc oncoprotein, a transcription factor that coordinates multiple aspects of the cell proliferation (cell cycle progression, cell growth, biogenesis and metabolism) with extracellular tissue remodeling programs like angiogenesis, migration and invasion. All of Myc's potentially potent tumorigenic aspects are restrained by the potent ability of Myc to trigger apoptosis in cells that stray from their orthotopic somatic niches. Use of a variety of reversibly switchable Myc transgenic mouse models has allowed us to define both the multiplicity of biological programs that Myc regulates *in vivo* and the role that apoptosis plays in limiting Myc oncogenic activity in differing tissues *in vivo*. Such studies underscore how complex tumor phenotypes arise from extremely simple combinations of mutation that,

minimally, drive cell proliferation and suppress the concomitant apoptosis. One key mediator of intrinsic tumor suppression is p53, Although p53 becomes inactivated in virtually all human cancer, it remains unclear whether the tumor suppressive action of p53 is mediated through its capacity to trigger apoptosis and/or growth arrest directly, in response to genotoxic damage or growth deregulation activation, or indirectly, through maintenance genome integrity and suppression of oncogenic mutations. To explore the role of p53 in tumor suppression *in vivo* we constructed a mouse in which the endogenous *p53* gene is modified to allow its function to be toggled reversibly between *p53* null and *p53* wild type states. This allows direct analysis *in vivo* of when, where and how p53 function is required to suppress neoplasia. One surprising conclusion from study of this model is that the DNA damage response appears irrelevant for p53-mediated tumor suppression *in vivo*. Separation of DNA damage and tumor suppression has far-reaching implications for our understanding of both tumor suppression and aging.



Samir Hanash
Fred Hutchinson Cancer Research Ctr.

Dr. Hanash leads a program in Molecular Diagnostics at the Fred Hutchinson Cancer Research Center. His major interest and expertise has been the application of genomics, transcriptomics and in particular proteomics to the molecular analysis of cancer to allow early detection and/or improved classification to individualize therapy. Dr. Hanash has led several program projects and large collaborative programs funded by the NIH, notably a collaborative study funded by the NCI for the molecular classification of adenocarcinomas, a consortium that he currently heads for application of proteomics to mouse models of cancer. Recently, Dr. Hanash was the PI on a study of the plasma proteome using multiple platforms, recently published in Nature Biotechnology and funded in part by support from seen NIH institutes. He is the inaugural president of the Human Proteome organization established to promote scholarly activities in the field of proteomics and to develop standards for the field.

Proteomics Approaches to Tumor Antigen Identification

There is increasing evidence for an immune response to cancer in humans, demonstrated in part by the identification of autoantibodies against a number of intracellular and surface antigens detectable in sera from patients with different cancer types. The identification of panels of tumor antigens that elicit an antibody response may have utility in cancer screening, diagnosis or in establishing prognosis Such antigens may also have utility in immunotherapy against the disease. There are several approaches for the detection of tumor antigens that induce an immune response. For most antigenic proteins that induce an antibody response in cancer identified using proteomics, post-translational modifications contributed to the immune response. However, these modifications are generally not captured using either recombinant proteins or antibodies that do not distinctly recognize specific forms of a protein. One approach for comprehensive analysis of proteins in their modified forms is to array proteins directly isolated from cells and tissues

following protein fractionation schemes. Proteins in reactive fractions are identified by mass spectrometry. The current status of application of this strategy to epithelial tumors will be presented.



Mary Hendrix
Northwestern University

Dr. Mary J.C. Hendrix received her B.S. degree in Biology/Pre-Med from Shepherd College (now called Shepherd University) in 1974, her Ph.D. in Anatomy from George Washington University in 1977, and an honorary D.Sc. in 1996 from Shepherd College. Dr. Hendrix was an NIH Postdoctoral Research Fellow at Harvard Medical School in the Department of Anatomy and Cell Biology from 1977 to 1980; Assistant, Associate and Professor (and Associate Head) at the University of Arizona from 1980-1993. She also served as the Kate Daum Research Professor, and Associate Director of Basic Research and Deputy Director for The Holden Comprehensive Cancer Center at The University of Iowa, for the Roy J. and Lucille A. Carver College of Medicine from 1996-2004. Currently, she serves as President and Scientific Director for the Children's Memorial Research Center Northwestern University Feinberg School of Medicine. She is the US Editor of Pathology Oncology Research, and Member of the Editorial Boards of Developmental Dynamics, Cancer Biology and Therapy, Journal of Cellular Biochemistry, Cancer Research, and the American Journal of Pathology. She is a Past-President of FASEB (Federation of American Societies for Experimental Biology) consisting of over 70,000 members, making it the largest coalition of biomedical research societies in the United States. She also serves on the National Advisory Council for the Human Genome Research Institute, the Annenberg Center for Health Sciences, the National Cancer Institute Board of Scientific Advisors, and on the Board of Directors for the Metastasis Research Society, and for Research!America. Her scientific objectives include identifying genes which contribute to cancer metastasis. Specific projects include signal transduction events initiated by cell adhesion molecules and growth factors; factors regulating interconversion of the tumor cell phenotype; regulation of matrix metalloproteinases by tumor and stromal cell interactions; tumor angiogenesis and vasculogenesis; role of the microenvironment in inducing and maintaining an aberrant cellular phenotype; and the identification of stem cell subpopulations within tumors.

Targeting the Plasticity of Metastatic Tumor Cells

Aggressive tumor cells share many characteristics with embryonic progenitors. During development, multipotent precursor cells undergo specific cell fate determination through the autocrine or paracrine delivery of signaling molecules. Similarly, during cancer progression, malignant cells release and receive cues that promote tumor growth and metastasis. In addition, the stem cell-like plasticity of aggressive tumor cells, particularly melanoma, is suggested by their molecular signature, which defines a dedifferentiated, multipotent plastic phenotype -- that can respond to microenvironmental factors and influence other cells through epigenetic mechanisms. To address the issue of tumor cell plasticity and to understand the clinical implications of targeting this phenotype, we employed an innovative experimental approach. We used the embryonic microenvironment of the zebrafish as a biosensor for tumor-derived signals. Using this model, we

show that multipotent, aggressive melanoma cells secrete Nodal (a potent embryonic morphogen) and consequently can induce ectopic formation of the embryonic axis. Most noteworthy, we show that Nodal is present in human metastatic tumors, but not in normal skin, and thus may be involved in melanoma pathogenesis. Inhibition of Nodal signaling reduces melanoma cell invasiveness, colony formation, and tumorigenicity. Interestingly, Nodal inhibition also promotes the reversion of melanoma cells toward a melanocytic phenotype concomitant with a diminution of the plastic phenotype. These data suggest that Nodal signaling plays a key role in melanoma cell plasticity and tumorigenicity, thereby providing a previously unknown molecular target for regulating metastatic tumor cell plasticity and progression.



Mien-Chie Hung
MD Anderson Caner Center

Dr. Mien-Chie Hung is Professor and Chair for the Department of Molecular and Cellular Oncology. He received his undergraduate and graduate degrees from the National Taiwan University in Taiwan and his Ph.D. from Brandeis University in Massachusetts. He was a postdoctoral fellow at the Whitehead Institute at MIT. He joined MD Anderson in 1986 as an Assistant Professor for the Department of Tumor Biology. He was later promoted to Associate Professor and Professor before serving as the department's Deputy Chair. He was the recipient of the Hubert L. and Olive Stringer Endowed Professorship from 1996 to 2000. In 2000, he was named to his current post. He also serves as the Director of the Breast Cancer Basic Research Program and is the Ruth Legett Jones Distinguished Chair. In addition to numerous publications and research endeavors, Dr. Hung has received several awards and honors, including the Faculty Achievement Awards in Education and in Basic Research, and the John P. McGovern Outstanding Teacher Award. In 2002, he became an Academician of the Academia Sinica in Taiwan. Dr. Hung serves as a founding Editorial Members on Cancer Cell as well as an Associate Editor on Cancer Research, Clinical Cancer Research, Molecular Cancer Research and Molecular Carcinogenesis.

Shuttling of Membrane Receptors and Cytoplasmic Molecules to the Nucleus: Importance to Tumor Progression

Previously, we have found that the rat neu, which is the rat version of human ErbB-2/HER-2 and a member of epidermal growth factor receptor (EGFR) family, was localized in the nucleus and could act as a transcriptional activator. Later, we demonstrated that the carboxy terminus of EGFR contains a strong transactivation activity and EGFR complexe recognizes an AT-rich consensus-sequence on cyclin D1 promoter. By using chromatin immunoprecipitation (ChIP) assays, we further showed that nuclear EGFR associated with promoter region of cyclin D1 *in vivo*. EGFR may function as a transcription factor to activate genes required for highly proliferating activities in the nucleus.

More recently, using the ChIP cloning strategy, we have identified that human HER-2/ ErbB-2 was associated with multiple genomic targets *in vivo*, including the cyclooxygenase enzyme *COX-2* gene promoter. We have shown that ErbB-2 forms a complex at a specific nucleotide sequence of the *COX-2* promoter and is able to stimulate its transcription. This study further strengthens the nucleocytoplasmic shuttling of ErbB-2 and demonstrates the functionality of ErbB-2 as a transcriptional regulator in the nucleus.

In addition, we have analyzed 130 breast carcinomas via immunohistochemical analyses for the levels of nuclear and non-nuclear EGFR. We found 37.7% of the cohort immunostained positively for nuclear EGFR and 6.9% with high levels of expression. Importantly, Kaplan-Meier survival analysis and log-rank test revealed a significant inverse correlation between high nuclear EGFR and overall survival ($P = 0.009$). Even the study from a small set of cohort, our findings indicate pathological significance of nuclear EGFR and may have important clinical implication. Moreover, we have demonstrated that EGFR physically interacts with signal transducers and activators of transcription 3 (STAT3) in the nucleus leading to transcriptional activation of inducible nitric oxide synthase (iNOS). In breast carcinomas, nuclear EGFR positively correlates with iNOS expression. Together, the study describes a mode of transcriptional control involving cooperated efforts of STAT3 and nuclear EGFR. Our work suggests de-regulated iNOS/NO pathway may partly contribute to the malignant biology of tumor cells with high levels of nuclear EGFR and STAT3. Most recently, we found that nuclear EGFR associates with and phosphoylates PCNA, an essential protein involved in DNA repair. Increased tyrosine phosphorylated PCNA coincides with pronounced cell proliferation and is correlated better than total PCNA with poor survival of breast cancer patients. Our results identify another novel function of nuclear EGFR. i.e. to control genomic integrity.



Scott E. Kern
Johns Hopkins University

Dr. Scott Kern, MD is a molecular biologist and gastrointestinal pathologist having a special interest in pancreatic cancer and its molecular defects. Discoveries in his laboratory included the first human Smad gene and their mutations in cancer, the first molecular mutation identified in BRCA2, the identification of mutations of the Fanconi Anemia genes BRCA2, FANCC, and FANCG in commercially available cancer cell lines, and the creation of pathway-reporter lines and somatic gene knockouts in cancer cells to facilitate the screening of compound libraries.

Hypersensitivities versus Reduction-of-fitness: Distinguishing Fact from Artifact in Pharmacogenomic Studies of Cancers having Specific Mutations

There are now ample opportunities to use genetically modified cell lines to discover new drugs against cancer. Unfortunately, there are also many artifacts in these models, which will eventually prevent the newly identified compounds from becoming clinically useful. Some artifacts, such as off-target effects, can usually be managed by the use of highly matched control lines. Others, such as clonal variation among

cell lines and the squelching of necessary intermolecular interactions by protein overexpression, are well-known and can be designed around, although with some difficulty. An under-recognized artifact is that caused by "loss of fitness", which is readily observed in transgenic animals (e.g., termination of fetal development) but can be difficult to recognize in cell lines. The cells of actual human tumors presumably experiment with new gene mutations that emerge repeatedly over decades within thousands of genetically distinct clones, selecting for the unique clone that can overcome the loss-of-fitness phenotype. Investigators, using deterministically engineered models, do not have this luxury, and the clones selected for testing of compound sensitivities can be atypical of the responses eventually observed in patients. Examples have been observed to affect studies of the p53-p21/SFN axis, the MKK4 gene, the BRCA2 gene, and other systems. In cancer cells made null for the SFN gene, which is activated by p53 upon DNA damage, DNA-damaging agents of many types cause a mitotic catastrophe, but p53-mutant tumor cells do not share this phenotype. In knockouts of the tumor-suppressor gene MKK4, experimental metastasis is suppressed rather than augmented as had been expected. In knockouts of BRCA2 in multiple species, cells are often hypoproliferative and the population may experience extinction, an experimental limitation that does not prevent patients from dying of BRCA2-mutant cancers. Experimental cells may, in similar ways, manifest drug hypersensities that are not shared among naturally occurring cancer cells. The loss-of-fitness phenotype may perniciously dominate some compound-screening strategies, resulting in ideally performing drug candidates for which the first experimental failures may be observed in a clinical trial.



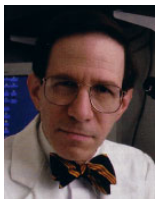
Tim MacLachlan
Genzyme Corporation

Tim MacLachlan received his Ph.D. in Genetics from Thomas Jefferson University under the mentorship of Antonio Giordano, studying new cell-cycle regulated kinases. Following this, he performed postdoctoral research under the supervision of Wafik El-Deiry at the Howard Hughes Medical Institute and the University of Pennsylvania investigating BRCA1 transcriptional activity. He joined Curagen Corporation in 2002, initiating the company's efforts with oncology drug discovery, and in 2005 he moved to Genzyme where he is currently leading a Pharmacology/Toxicology group focused on monoclonal antibodies and gene therapy.

TGF-beta Neutralizing Antibody GC1008 for Melanoma and Renal Cancer

The activity of the TGF-β signaling pathway in cancer has been under investigation for many years. While it mostly has been implicated in tumor suppression, particularly in colon cancer, TGF-β can also act to foster a metastatic transition. In the interest of inhibiting this function, we have developed a fully human monoclonal antibody directed against all isoforms of TGF-β, called GC1008. The rodent surrogate of GC1008, 1D11, possesses very specific activity in preclinical cancer models. However, as expected, broad blockade of TGF-β function is not without its unintended consequences. The path forward in dealing with these findings,

and their implication in the ongoing Phase I clinical trial in melanoma and renal cancer, will be discussed.



Sanford Markowitz, M.D., Ph.D.
Case Western Reserve University

Sanford Markowitz is the Ingalls Professor of Cancer Genetics at Case Western Reserve University and is an Investigator of the Howard Hughes Medical Institute. Dr. Markowitz received his B.A. at Harvard College, followed by his M.D. and Ph.D. at Yale University. He received clinical training in internal medicine at the University of Chicago followed by a fellowship in medical oncology at the National Cancer Institute. Dr. Markowitz's research group focuses on translational studies of human colon cancer. Contributions from Dr. Markowitz's group include the discovery of TGF-beta receptor mutations in human cancers, including colon cancer, the discovery that a specific sequence in TGF-beta receptors becomes ubiquitously activated as a target for frameshift mutations in colon cancers with mismatch repair deficiency, and the finding that E-cadherin promoter methylation provides the second hit in the development of cancer in individuals with the syndrome of hereditary diffuse gastric cancers. More recent findings will be discussed at this meeting.

Genetic Lessons from Colon Cancer

Colon cancer is the second leading cause of cancer death among adult Americans. The majority of cancers of the colon and stomach develop mutations that inactivate TGF-beta signaling. Most commonly this is accomplished via either mutations that inactivate the type II TGF-beta receptor (RII) or via mutations that inactivate the Smad4 transcription factor that is a post-receptor element in the TGF-beta signaling pathway. Recent studies from my laboratory demonstrate that one of the mechanisms by which TGF-beta suppresses human colon cancer development is by negative regulation of the COX-2 oncogene. Specifically, whereas expression and activity of COX-2 is increased as an early event in colon neoplasia, and serves to elevate production of mucosal prostaglandins; we find that TGF-beta potently induces colonic expression of 15-Prostaglandin Dehydrogenase (15-PGDH), an enzyme that inactivates and degrades prostaglandins. Whereas 15-PGDH is strongly expressed in the luminal portion of normal human colonic mucosa, it is ubiquitously absent in human colon cancers. Restoration of 15-PGDH expression in colon cancer cell lines abrogates the ability of these cells to grow and form tumors in athymic mice, directly demonstrating the ability of this enzyme to mediate a tumor suppressor function. Moreover, the 15-PGDH knockout mouse demonstrates marked susceptibility to colon tumor induction, with the 15-PGDH knockout allele conferring a 7-fold increase in colon tumor numbers when combined with the germline APC mutant Min allele, and with the 15-PGDH knockout allele abrogating the normal resistance of the C57BL6J mouse to colon tumor induction by the carcinogen AOM. Microscopic examination shows that the wild-type C57BL6J mouse when treated with AOM develops microscopic aberrant crypt foci, but that these lesions are hyperplastic and do not progress. In contrast, the 15-PGDH knockout mouse develops aberrant crypt foci that morphologically are dysplastic, that show marked induction of nuclear cyclin

D1 expression, that show aberrant expression of the Ki-67 proliferation marker in the normally non-dividing upper portions of the colon crypt, and that progress to frank tumors. Strategies for reactivation of 15-PGDH thus provides a potentially new approach for colon cancer chemoprevention.

A second focus of the Markowitz laboratory has been the development of molecular approaches for non-invasive early detection of colon cancer. Recently, our group has shown high sensitivity and specificity for detection of early stage colon cancer through detection of aberrantly methylated vimentin gene sequences shed into the feces of colon cancer patients. This assay is currently being developed for clinical use.

David McConkey
MD Anderson Cancer Center

Exploiting Endoplasmic Reticular Stress in Cancer Therapy

Bortezomib (PS-341, Velcade) is a dipeptidyl boronic acid inhibitor of the 26S proteasome that was developed for use in cancer therapy. We recently presented evidence that bortezomib induces apoptosis in human pancreatic cancer cell lines and in orthotopic tumor xenografts by inducing an unorthodox form of endoplasmic reticular (ER) stress. Bortezomib interfered with the unfolded protein response (UPR) by inhibiting the activation of PKR-like ER kinase (PERK) and phosphorylation of eIF2α. The buildup of ubiquitylated proteins in the face of ongoing protein synthesis resulted in the activation of procaspase-4, an initiator caspase that we demonstrate is localized to the ER, and peptide- or siRNA-mediated inhibition of caspase-4 blocked bortezomib-induced cell death. Ultrastructural analysis of tumor cells exposed to bortezomib revealed that they contained aggregates of electron-dense, ubiquitinated proteins (aggresomes) that appeared as discrete structures that abutted the nuclear envelope. Aggresomes were observed in 6/8 of human pancreatic cancer cell lines tested, and they were only observed in cells that were negative for active caspase-3 or TUNEL staining. They were also formed in orthotopic tumors in animals treated systemically with bortezomib but were not detected in adjacent normal pancreatic epithelial cells. Importantly, siRNA directed against histone deacetylase-6 (HDAC-6) disrupted aggresome formation and sensitized cells to bortezomib-mediated cell death. Furthermore, chemical HDAC inhibitors (trichostatin A, SAHA) disrupted aggresome formation and synergized with bortezomib to induce apoptosis in pancreatic cancer cells in vitro and in orthotopic xenografts in vivo, leading to increased inhibition of tumor growth without excessive toxicity. Interestingly, immortalized pancreatic ductal epithelial cells (HPDEs) displayed lower basal rates of protein synthesis than tumor cells and failed to form aggresomes or undergo apoptosis in response to bortezomib. Transfection with mutant (active) K-ras increased bortezomib sensitivity in these cells, effects that were linked to activation of the mTOR-$P70^{RSK}$ pathway and increased translation. Conversely, mTOR was constitutively active in almost all human pancreatic cancer lines, and the mTOR inhibitor rapamycin reduced basal

translation and sensitivity to borteomzib in these cells. Our data implicate oncogene-mediated increases in translation in the sensitivity of pancreatic cancer cells to bortezomib and support the development of combination regimens that exploit bortezomib's effects on ER stress.



Spyro Mousses
TGen

Dr. Spyro Mousses is the director of the Pharmaceutical Genomics Division of the Translational Genomics Research Institute (TGen) in Arizona. He has pioneered a convergence of scientific technologies to identify contextual vulnerabilities that could be exploited for drug discovery and to guide drug development. Dr. Mousses has invented a cellular pharmacogenomic approach for genome wide analysis of the causal role that genes play in response to drug exposure. His laboratory has established an extensive infrastructure for the high throughput analysis of small compounds and siRNA in cell based assays combined with whole genome and transcriptome molecular profiling.

Dr. Mousses graduated from the University of Toronto in 1998. He worked as a Staff Scientist at the NHGRI/NIH and joined TGen in 2002. Dr. Mousses moved his Cancer Drug Discovery lab from Gaithesburg, MD to the Mayo Scottsdale campus, which he still heads today within the Pharmaceutical Genomics Division of TGen.

Application of Novel Cellular Genomics Strategies to Support Cancer Drug Development

Genome wide profiling of gene states (structure, expression, etc.) has emerged as a means for molecularly defining disease. The next challenge is to match the specific molecular context of disease with the most appropriate therapy. Systems medicine is emerging as a new field of research which seeks to identify contextual vulnerabilities that arise in disease states in order to link the right drug therapy to the right disease context. Towards this end, TGen has developed and applied a number of genome wide and genome compatible technologies and strategies to both profile the disease context (structural and functional genomics), as well as profile context selective drug targeting (cellular genomics). Our group has focused primarily on cellular genomics using very high throughput RNAi to systematically knockdown genes across the genome, and determine the functional role of each gene in various cellular process including cell growth, survival, molecular endpoints and drug response. The computational 'fusion' of multidimensional data sets to integrate structural, functional and cellular genomic information has proven to be a very powerful strategy for generating new predictive models of context dependent targeting. In oncology, this research has led to the discovery of context specific vulnerabilities, enabling us to prioritize pharmacologically and clinically relevant drug targets and supports more personalized medicine oriented drug discovery. Additionally, we have shown that this approach can lead to the discovery of genes that are causally involved in determining specific response to cancer drugs. These genes are currently being advanced as candidate predictive markers to select patient populations that would respond to specific cancer drugs, and as putative combination targets to enhance response

in patients that are not responding. Such information can improve and accelerate the clinical development of emerging cancer therapies. This strategy represents a new paradigm for both drug discovery and drug development, and has the potential to some day impact on how therapeutic decisions are made.



Ben Neel
BIDMC, Harvard University

Dr. Neel carried out his graduate work on the mechanism of oncogenesis by slowly transforming RNA tumor viruses with Dr. William S. Hayward at the Rockefeller University, receiving his Ph.D. in 1982. After receiving his M.D. from Cornell University Medical College, he completed medical internship and residency training at Beth Israel Hospital in Boston. He subsequently carried out post-doctoral work in Dr. Raymond Erikson's laboratory at Harvard University, before returning to Beth Israel Hospital and Harvard Medical School as Assistant Professor of Medicine in 1988. He is currently the William B. Castle Professor of Medicine and Director of the Cancer Biology Program at Beth Israel-Deaconess Medical Center. His laboratory focuses on cellular signaling, particularly by members of the protein-tyrosine phosphatase super-family, particularly Shp2, which was identified by his group in the early 1990s. He is the author of over 120 primary papers and multiple reviews on the structure and function of PTPs, and serves as an Editor of Molecular and Cellular Biology, and on the editorial boards of several other journals. He also was the first recipient of the Gertrude Elion Award of the AACR. In 2007, Dr. Neel will become Director of the Ontario Cancer Institute in Toronto, CA and Professor of Medical Biophysics at the University of Toronto.

The Shp2/Ras/Erk Pathway in Human Disease and Cancer

Shp2 (PTPN11) is a ubiquitously expressed non-receptor tyrosine phosphatase that plays a positive regulatory role in receptor tyrosine kinase (RTK) and cytokine receptor signaling. Shp2 is required for activation of the Ras/Erk pathway, whereas it has variable effects on other downstream signaling cascades (e.g., PI3K/Akt, NF-kB) in different contexts. In the absence of a phosphotyrosyl (pTyr) peptide ligand for its N-SH2 domain, Shp2 is inactive state, due to an intramolecular inhibitory interaction between the N-SH2 and PTP domains. Ligand binding disrupts auto-inhibition, resulting in marked enzyme activation. Consequently, recruitment of Shp2 either directly to receptors or to scaffolding adapters such as Gab family proteins normally is tightly coupled to catalytic activation. Engineered mutations of key contact residues between the N-SH2 and PTP domains can generate "activated mutants" that exhibit increased enzymatic and biological function. Germ line mutations in Shp2 cause ~50% of cases of Noonan syndrome (NS), an autosomal dominant disorder characterized by facial dysmorphia, short stature, cardiac defects and an increased incidence of certain childhood leukemias. Somatic mutations in Shp2 are found in sporadic leukemias, including Juvenile Myelomonocytic Leukemia (JMML), CALLA+ B-ALL, and adult AML, and rarely in solid tumors. These mutations typically affect residues involved in basal inhibition, and biochemical and biological analyses, including mouse modeling experiments, suggest that these human Shp2 mutants are similar to the

engineered "activated mutants" generated previously. Although Shp2 mutations in solid tumors are rare, some tumors over-express Shp2 binding proteins; e.g., Gab2 is over-expressed in a significant percentage of human breast tumors and may accomplish the same end as Shp2 mutations, albeit by different means. We have generated mouse models of Shp2-induced leukemias, and will review the current status of our understanding of the pathogenesis of this disorder. The role of mutations in other Ras/Erk pathway members in human developmental disorders and cancer also will be discussed.



William G. Nelson
Johns Hopkins University

Dr. Nelson completed his M.D. and Ph.D. training at the Johns Hopkins University School of Medicine, and then pursued Internal Medicine residency training and Medical Oncology fellowship training at the Johns Hopkins Hospital. Now a Professor of Oncology, Urology, Pharmacology, Medicine, Pathology, and Radiation Oncology at the Johns Hopkins University School of Medicine, with a Joint Appointment in Environmental Health Sciences at the Johns Hopkins University Bloomberg School of Public Health, Dr. Nelson directs a research laboratory focused on discovering new strategies for prostate cancer treatment and prevention, and manages a clinical practice focused on developing these new treatment and prevention approaches in early "proof-of-principle" prostate clinical trials. Within the Sidney Kimmel Comprehensive Cancer Center, Dr. Nelson serves as the Associate Director for Translational Research and the Co-Director of the Prostate Cancer Program. He is also the Principal Investigator for the National Cancer Institute (NCI)-funded Molecular Targets Training Program, dedicated to providing clinical oncology fellows specific training in translational research, and for the NCI-supported Prostate Cancer SPORE Program, a multidisciplinary research effort for translational research targeting new approaches to prostate cancer detection, diagnosis, prevention, and treatment.

New Approaches to the Reactivation of Epigenetically Silenced Genes in Cancer Cells

Hypermethylation of CpG island sequences at the regulatory regions of key cancer genes constitutes the earliest and most common somatic genome change in epithelial cancers. For example, several gene loci, including *GSTP1*, *RASSF1a*, *APC*, *PTGS2*, *MDR1*, *EDNRB*, *ESR1*, *CDKN2a*, and *hMLH1*, are known to be affected by CpG island hypermethylation in prostate cancer cells. Hypermethylation at the *GSTP1* CpG island has also been detected even in early prostate cancer precursor lesions, such as proliferative inflammatory atrophy (PIA) and prostatic intraepithelial neoplasia (PIN). Increased CpG dinucleotide methylation at these genes results in transcriptional silencing, typically mediated by *trans*-repressors such as $^{5\text{-m}}$CpG-binding domain (MBD) family proteins. However, unlike gene mutations or deletions, in which the coding sequence of DNA has been irreversibly altered, CpG island hypermethylation, in which the DNA sequence is preserved, is a somatic genome alteration that may be targeted for rational prostate cancer prevention and treatment. One approach to reversing CpG island hypermethylation focuses on inhibition of the DNA methyltransferases

(DNMTs) responsible for maintenance of CpG dinucleotide methylation patterns during DNA replication, using agents such as 5-aza-cytidine, 5-aza-deoxycytidine, zebularine, procainamide, or hydralazine, in an attempt to reduce $^{5\text{-m}}$CpG density at the CpG island sequences. Unfortunately, the use of the nucleoside analog DNMT inhibitors, which incorporate into genomic DNA, is associated with side effects, such as low blood counts, and may carry a risk of causing mutations. Non-nucleoside DNMT1 inhibitors, which do not incorporate into genomic DNA, may be safer drugs. Another strategy for restoring the expression of genes silenced by somatic CpG island hypermethylation in prostate cancer, and in other cancers, features the selective targeting of MBD family proteins that mediate $^{5\text{-m}}$C-dependent transcriptional repression. Of the MBDs, MBD2 has emerged as a tantalizing new target for cancer prevention drugs: *(i)* MBD2 selectively binds the *GSTP1* CpG island when it is methylated, *(ii)* siRNA-mediated reduction in MBD2 levels activates *GSTP1* expression despite CpG island hypermethylation, *(iii)* $Apc^{Min/+}Mbd2^{-/-}$ mice develop far fewer intestinal adenomas, and survive longer, than do $Apc^{Min/+}Mbd2^{+/-}$ or $Apc^{Min/+}Mbd2^{+/+}$ mice, and *(iv)* $Mbd2^{-/-}$ mice maintain both normal gene imprinting and repression of endogenous retroviral sequences, and do not display ectopic gene expression. "High-throughput" screening of chemical libraries has identified MBD2 antagonists, new candidate drugs for the prevention and treatment of prostate cancer and other epithelial cancers.



Nicholas C. Nicolaides
Morphotek, Inc.

Nicholas C. Nicolaides Ph.D. is the President, Chief Executive Officer and a co-founder of Morphotek. Prior to founding Morphotek, he was the Director of Research at Magainin Pharmaceuticals Inc. Dr. Nicolaides is a trained molecular geneticist and has authored a number of scientific papers on the molecular and genetic basis of cancer and respiratory diseases, antibody engineering, and cellular evolution. Dr. Nicolaides received his B.S. in Biology at St. Joseph's University, Philadelphia, PA, his Ph.D. in human genetics at Thomas Jefferson University, Philadelphia, PA and continued his post-doctoral training in human molecular genetics at Johns Hopkins University, Baltimore, MD. While at Johns Hopkins, his research resulted in the discovery of the genetic cause of Hereditary Non-Polyposis Colon Cancer and the development of Morphotek's whole genome evolution technology called morphogenics. Morphotek's technology is a proprietary platform that can enhance the natural process of genetic evolution within a targeted cell host to yield genetically diverse sib cells that are suitable for pharmaceutical product development in the areas of antibody and protein discovery and optimization. Morphotek is applying its technology to develop therapeutic antibodies for the treatment of cancer, inflammation and infectious diseases. The Company has a number of antibody-based therapies in clinical trials and preclinical development.

Whole Genome Evolution Technology: Development of Therapeutic Antibodies Specific for Cancer Pathways

MORPHODOMA® technology is a platform process that enhances the activity of therapeutic monoclonal antibodies and attendant cell lines for

their scaleable manufacturing via whole genome evolution. The process involves the reversible regulation of the DNA mismatch repair system using proprietary small molecules or polypeptides resulting in randomized inheritance of mutations in genes encoding for antibodies and/or pathways involved in overall higher productivity. We have employed MORPHODOMA technology to develop a portfolio of therapeutic antibodies derived by *ex vivo* immunization of human B-cells to disease-specific target antigens as well as from optimized chimeric or humanized antibodies. MORAb-003 is a humanized antibody that targets the folate receptor alpha (FRA), a glycosylphosphatidylinisotol (GPI) anchored cell surface receptor over-expressed on the majority of epithelial ovarian cancers. The antibody was originally discovered from a panel of monoclonal antibodies derived from mice immunized with human tumor cells. Sequences derived from the mouse antibody were engineered onto a human IgG_1 backbone in an attempt to generate a functional humanized antibody. This antibody appeared to have a lower binding affinity to FRA than the mouse precursor. The humanized antibody was evolved via MORPHODOMA technology to yield, MORAb-003, a high-affinity humanized mAb.

Folate receptor alpha exhibits many of the same biological properties of an oncogene. It is transforming when over-expressed in cells and is associated with aggressive forms of human cancers. The MORAb-003 antibody has bimodal anti-tumor activity on FRA^+ tumors. It can prevent phosphorylation of substrates specific for lyn kinase and suppress proliferation of cells over-expressing FRA. In addition, MORAb-003 is highly effective in eliciting FRA^+ tumor cell killing via antibody dependent cellular cytotoxic (ADCC) and complement dependent cytotoxic (CDC) mechanisms and suppression of FRA^+ tumor growth in rodent xenograft models. The antibody has been tested in a Phase I clinical trial with patients diagnosed with platinum-resistant epithelial ovarian cancer (EOC) and is currently in Phase II trials in patients with platinum-sensitive disease. In EOC, FRA is over-expressed in greater than 90% of tumors, a finding suggestive of a positive selection for this pathway in the natural progression of the disease. To date, MORAb-003 has been well tolerated in both clinical settings and has shown signals of efficacy as determined by CA-125 biomarker responses and tumor growth measurements via RECIST criteria. Data will be presented describing MORPHODOMA applications to develop MORAb-003, biochemical properties of the FRA pathway and clinical data of patients treated with MORAb-003.



Kornelia Polyak
DFCI, Harvard University

Dr. Polyak obtained her M.D. degree in 1991 from the Albert Szent-Gyorgi Medical School in Szeged Hungary and her Ph.D. degree in 1995 from Cornell University Graduate School of Medical Sciences/Sloan-Kettering Cancer Center, New York. Dr. Polyak completed her postdoctoral training in Baltimore at the Johns Hopkins Oncology Center in the laboratory of Drs. Bert Vogelstein and Ken Kinzler. Dr. Polyak joined the faculty of Dana-Farber Cancer Institute and Harvard Medical School in 1998 as Assistant Professor of Medicine and she has been an

Associate Professor since 2006. The focus of her laboratory is to investigate the molecular basis of breast cancer initiation and progression with special emphasis on the role of the microenvironment and stem cells in these processes. Her work is focusing on identifying molecular alterations between normal and cancerous breast tissue using various technologies, determining their consequences, and utilizing them to improve the clinical management of breast cancer patients. Dr. Polyak have received several awards including the Julienne Rachele Prize (1995, Cornell University), the W. Barry Wood, Jr. Research Prize (1998, Johns Hopkins University), Kimmel Scholar Award (1999, Sidney Kimmel Foundation), V Scholar award (2001, V Foundation), the Tisch Family Outstanding Achievement Award (2005, Dana-Farber Cancer Institute), and the Claire W. and Richard P. Morse Research Award (2006, Dana-Farber Cancer Institute).

Molecular Profile of Breast Stem Cells

The existence of cells with distinct phenotypes including stem cell-like properties in normal human mammary epithelium and breast carcinomas has been proposed. However, detailed molecular characterization of these cells has not been accomplished, and their clinical relevance is unclear. To address these issues we determined the gene expression and genetic profiles of distinct cell populations purified from breast carcinomas and normal breast tissue using cell surface markers CD24 and CD44 that have been associated with stem cell-like properties. Normal CD44+ and differentiated CD24+ luminal epithelial and CD10+ myoepithelial cells were also analyzed using MSDK to delineate DNA methylation changes that may underlie cellular differentiation. The CD24+ or CD44+ cell populations from the same tumor were clonally related but not always identical, and epigenetically distinct. A gene signature specific for CD44+ cells was enriched for known stem cell markers and was associated with decreased overall and relapse free survival in lymph node negative breast cancer patients. Systemic network analyses determined that the TGF-β pathway is specifically active in CD44+ breast cancer cells. Consistent with this, TGFBR2 was only expressed in these cells and its inhibition induced a more epithelial phenotype. Our data suggest prognostic relevance of CD44+ cells and tumor heterogeneity and imply therapeutic targeting of distinct tumor cell subpopulations.



Martin G. Pomper
Johns Hopkins University

Martin Pomper is an associate professor in Radiology, Pharmacology & Molecular Sciences and Oncology at Johns Hopkins University. He received his undergraduate, graduate and medical degrees from the University of Illinois at Urbana-Champaign in 1982, 1989 (Ph.D., chemistry with Katzenellenbogen) and 1990, respectively. His postgraduate medical training was at Johns Hopkins and included internship (Osler Medical Service), residencies (diagnostic radiology and nuclear medicine) and fellowship (neuroradiology). He is board-certified in diagnostic radiology and nuclear medicine. He has been on the Radiology faculty at Johns Hopkins since 1996. He is currently the director of the Johns Hopkins Small Animal Imaging Resource Program and associate director of the In Vivo Cellular and Molecular Imaging Center, both of which are funded by the NCI to support molecular imaging research. His interests are in the development of new

radiopharmaceuticals, optical probes and techniques for molecular imaging of cancer and CNS disease. His research group consists of chemists, physicists, molecular biologists and clinicians working together toward clinical molecular imaging. He currently serves as Treasurer of the Society for Molecular Imaging and President of the Society of Nuclear Medicine's Molecular Imaging Center of Excellence.

Translational Molecular Imaging for Oncology

Although most clinical diagnostic imaging studies employ anatomic techniques such as computed tomography (CT) and magnetic resonance (MR) imaging, much of radiology research currently focuses on adapting these conventional methods to physiologic imaging as well as on introducing new techniques and probes for studying processes at the cellular and molecular levels *in vivo*, i.e. molecular imaging. Molecular imaging promises to provide new methods for the early detection of disease and support for personalized therapy. Although molecular imaging has been practiced in various incarnations for over 20 years in the context of nuclear medicine, other imaging modalities have only recently been applied to the noninvasive assessment of physiology and molecular events. Nevertheless, there has been sufficient experience with specifically targeted contrast agents and high-resolution techniques for MR imaging and other modalities that we must begin moving these new technologies from the laboratory to the clinic. This brief overview will outline molecular imaging from probe development to clinical translation, with a focus on translational (small animal) and early clinical imaging of cancer. We will discuss the ability for molecular imaging to assess specific signal transduction cascades, which are increasingly the targets of newer, cytostatic therapeutic agents, and provide examples of how existing or readily accessible molecular tracers and techniques can provide insight into rather complex biological phenomena *in vivo*.

Vivek Rangnekar
University of Kentucky

Dr. Vivek M. Rangnekar is Professor and Alfred Cohen, M.D., Chair in Oncology Research at the University of Kentucky, College of Medicine, in Lexington, KY. He also serves as Associate Director of the Markey Cancer Center, and Chief of the Division of Radiation Biology at the University of Kentucky. He has participated on several NIH and DOD study sections. His laboratory first identified the Par-4 gene in prostate cancer cells and described its functional role and mechanism of cancer selective apoptosis. Subsequently, a number of research teams world-wide have worked on Par-4 and extended the findings to various cancer models, as well as to apoptosis in neuronal degeneration and depression. His pioneering work on Par-4 has been widely acclaimed and his research program is supported by several grants from the National Institutes of Health over the past 15 years.

Strategies for Modification of Host Stroma for Resistance to Tumor Growth

Ectopic over-expression of Par-4 induces apoptosis in cancer cells but not in normal cells. The core domain of Par-4, designated the SAC domain, controls the cancer-selective action of Par-4. Ectopic over-expression of the SAC domain selectively induces apoptosis of cancer

cells, not normal cells. Moreover, intra-tumoral injection of an adenovirus expressing the SAC domain causes regression of a broad range of non-autochthonous tumors in nude mice. As there is ample evidence in literature to support the notion that host stroma supports progression of tumor growth, we sought to determine whether genetic modification of the host stroma for expression of the SAC domain can confer resistance to tumor growth. Accordingly, we made transgenic mice that ubiquitously express the SAC domain under control of the actin promoter and CMV enhancer. The transgenic mice were embryonically and developmentally normal. Moreover, the average life span of the SAC-transgenic mice was longer than that of littermate control mice or GFP-producing transgenic mice, which develop spontaneous tumors at a relatively higher frequency. Offspring from crosses of the SAC-transgenic mice with tumor prone TRAMP mice impeded the growth of autochthonous prostate adenocarcinoma. Unexpectedly, the SAC-transgenic mice were resistant to the growth of non-autochthonous tumors derived from Lewis lung carcinoma cells and prostate cancer cells. The mechanism underlying resistance to tumor growth/metastasis in the SAC-transgenic mice indicates that the SAC-domain offers a unique opportunity for modification of the host stroma to inhibit tumor growth.



E. Prem Reddy
Fels Institute

Dr. E. Premkumar Reddy obtained his Ph.D. in Molecular Biology in 1971 and after post-doctoral training in UCLA School of Medicine and NCI, he joined the National Cancer Institute, with which he was associated till 1984. In 1984, he moved to Hoffmann La Roche and held appointments both at Hoffmann La Roche and the Roche Institute of Molecular Biology as a Full Member. In 1986, he moved to the Wistar Institute and became the Deputy Director of the Institute. In 1992, he was appointed as the Director of the Fels Institute for Cancer Research and Molecular Biology of Temple University. Dr. Reddy served as a member of the Board of Directors of NIEHS from 1990-1995. He the founding and current Editor of the cancer journal Oncogene.

The most notable of Dr. Reddy's findings are the first molecular cloning and sequence determination of a number of oncogenes, which include, mos, myb, myc, abl, fgr and sis. He was also one of the first to demonstrate that ras genes are activated in human cancers by point mutations. His basic work centers around the role of nuclear oncogenes, cell cycle and apoptotic genes in cancer progression and as targets for the development of novel cancer drugs. His translational research centers around the development of targeted drugs that specifically attack tumor cells while sparing normal cells from any collateral damage. According to the Institute of Scientific Information, Dr. Reddy is amongst the top 0.5% of the most highly cited authors in the world.

Targeting the Cell Cycle Machinery for Cancer Therapy

Cyclin dependent kinases (Cdks) and their cyclin subunits regulate cell cycle progression of cells through G0/1 through M phases. Alterations in cell cycle regulators such as Cdks, cyclins and Cdk inhibitors can result in disorded and the uncontrolled cell growth that leads to development of cancer. Among the Cdks, Cdk2 plays an important role

in G1-S transition and for S-phase progression. Recent reports indicate that deregulation of Cdk2 may play an important role in melanoma development and hence inhibitors of this kinase might be useful therapeutic agents for this form of cancer. In this study we describe the profile of ON04070, a non-ATP competitive inhibitor of Cdk2 activity, with an IC_{50} of 76 nM. This inhibitory activity was found to be specific for Cdk2 and this compound did not exhibit cross inhibitory activity with other Cdks such as Cdk4, Cdk1, Cdk8 and Cdk9. Treatment of SKMEL-28 cells with ON04070 leads to a significant delay in cell cycle progression followed by a G2 arrest. This growth arrest is accompanied by a reduction in levels of phosphorylated Retinoblastoma protein $p105^{Rb}$ on residues S-780 and S-807/811. Subsequent to treatment with ON04070, cells also increased their expression of the CDK-2 inhibitor, p21. ON04070 treatment of cultured melanoma cells also induces dose-dependent apoptosis, based upon our observation of increased levels of cleaved caspase-3 and Poly (ADP-Ribose) polymerase (PARP). Interestingly, ON04070 treatment also results in decreased levels of CDK-4, CDK-6 and CDC-25C. Taken together, these results provide an early foundation upon which to build treatment protocols for melanoma in the pre-clinical setting.



William Sellers
Novartis

Dr. Sellers, is VP and Global Head of Oncology at the Novartis Institutes of BioMedical Research. In this capacity he leads the drug discovery efforts focused in oncology. Dr. Sellers received his B.S. from Georgetown University in 1982 and his M.D. from the University of Massachusetts Medical School in 1986. He completed internship and residency training in Internal Medicine at the University of California San Francisco in 1989 and trained in Medical Oncology at the Dana-Farber Cancer Institute where he became an Associate Professor of Medicine. His lab at the Dana-Farber Cancer Institute focused on defining the molecular and genetic basis for prostate cancer and on exploiting the defining events for therapeutic purpose. This work focused on understanding the molecular mechanisms of growth regulation of the PTEN tumor suppressor gene. Towards the goal of comprehensive elucidation of genetic alterations in cancer, together with Matthew Meyerson, they initiated efforts to resequence large numbers of genes in diverse cancer types and to use high-density SNP arrays for defining LOH and copy number alterations across a broad spectrum of cancer types. This work led to discovery of EGFR mutations in lung adenocarcinoma and the discovery of the oncogenic role of the MITF gene in melanoma.

From Somatic Genetic Alterations in Cancer to Therapeutic Intervention

Somatic genetic events are linked to the initiation and progression of cancer. Over the past decade it has become clear that such somatic alterations also establish cancer dependence that can be exploited for therapeutic gain. Our group has approached the systematic identification of somatic changes in the genomes of primary tumors and cancer cell lines using high-density SNP arrays to characterize structural alterations and high-throughput exon resequencing to identify nucleotide level alterations. These efforts have led to the identification of activating mutations in EGR in lung adenocarcinoma.

Using high-density SNP arrays and integrated expression analysis to interrogate the NCI60 panel of cancer cell lines we have identified gene amplification of the microophthalmia transcription factor (MITF) in malignant melanoma. Such amplification is associated with poor survival in metastatic melanoma. MITF is a known master regulator of the melanocyte lineage and thus the gene amplification event appears to maintain a normal requirement of MITF for melanocyte survival. Thus, MITF amplification appears to be a manifestation of lineage addiction. Together with BRAF V600E, MITF is capable of transforming immortalized human melanocytes.

Finally, attempting to link directly genetic alterations to therapeutic dependence, we have developed in silico supervised analysis methods for interrogating the NCI60 chemosensitivity data. Together with the Rosen lab, we found that BRAF mutant cell lines were exquisitely sensitive to MEK inhibition while RAS mutant cell lines were not.

Together these data suggest that systematic approaches exploiting genome-scale data sets can unveil therapeutically tractable cancer dependencies.



Jeffrey Settleman
MGH, Harvard University

Dr. Settleman is among the research leaders in the area of signal transduction pathways mediated by small GTPases and kinases. Dr. Settleman conducted his Ph.D dissertation research at Yale University and was a post-doctoral with Robert Weinberg at MIT. He joined the Harvard School of Medicine faculty in 1992. Dr. Settleman is currently a Professor of Medicine at Harvard Medical School and Director of the Center for Molecular Therapeutics at the Massachusetts General Hospital Cancer Center. His work makes use of mouse and Drosophila as model organisms to study signaling pathways that contribute to tissue morphogenesis during development, and he uses cultured human cancer cell lines to study signaling pathways that determine tumorigenesis and response to therapy. Dr. Settleman was involved in the recent discovery of EGF receptor mutations in lung cancer that correlate with clinical response to EGF receptor inhibitory drugs. He has also contributed to studies that correlated amplification of the MET tyrosine kinase with sensitivity to a MET-selective kinase inhibitor in a subset of gastric cancer cell lines. Dr. Settleman has recently used cell culture models of oncogene dependency to identify a novel mechanism to explain the phenomenon of "oncogene addiction'.

EGF Receptor Mutants in Human Lung Cancer

We have recently identified a novel class of somatic activating mutations within the epidermal growth factor receptor (EGFR) gene in human lung tumors. These mutations are detected in 10-30% of non-small cell lung cancers and they have been correlated with dramatic clinical responses to the selective EGFR kinase inhibitors gefitinib (Iressa) and erlotinib (Tarceva) in a subset of treated patients. The EGFR mutations are clustered in specific sites within the ATP pocket of the kinase domain, and they affect both EGFR-mediated signaling as well as sensitivity to EGFR inhibitory drugs. Specifically, mutant receptors exhibit quantitatively and qualitatively altered signaling

output that leads to the engagement of cell survival pathways on which tumor cells are dependent. These mutant kinases also exhibit ~10-fold increased sensitivity to inhibition by gefitinib and erlotinib. In lung cancer patients with EGFR mutations, clinical responses are rapid and dramatic, but the average duration is only ~6 months, suggesting that acquired drug-resistance will limit the utility of this therapy. We have identified several mechanisms by which resistance to drug treatment can arise. These studies have begun to shed light on mechanisms underlying primary and secondary resistance to targeted EGFR inhibitors, and have led to the identification of a distinct class of EGFR inhibitors that may be effective in the clinical management of lung tumors that have become resistant to these drugs. In addition, our studies of "oncogene addiction" have led to a novel hypothesis regarding the mechanism by which tumor cells are killed in response to molecularly targeted drug treatments. This new mechanism, which relates to the kinetics of decay of pro-apoptotic and pro-survival signals in tumor cells upon acute inactivation of an oncogene, is referred to as "oncogenic shock", and may have implications for strategies to target human cancers therapeutically.



Frank Slack
Yale University

Frank Slack received his B.Sc from the University of Cape Town in South Africa, before completing his Ph.D in molecular biology at Tufts University School of Medicine. He started work on microRNAs as a postdoctoral fellow in Gary Ruvkun's laboratory at Harvard Medical School, where he co-discovered the second known microRNA, let-7. He is currently an Associate Professor in the Department of Molecular, Cellular and Developmental Biology at Yale University. The Slack laboratory studies the roles of microRNAs and their targets in development and disease, particularly cancer.

Oncomirs - microRNAs and Cancer

Mis-regulation of genes that control cell proliferation and cell fate determination during development often contributes to cancer. In C. elegans, let-7 controls the timing of seam cell terminal differentiation during epidermal cell development. In let-7 mutants, cells frequently fail to terminally differentiate, and instead elect to divide again, a hallmark of cancer. let-7 encodes a microRNA (miRNA), which are regulatory RNAs found in multicellular eukaryotes. In humans they are implicated in cancer. We have found that C. elegans let-7 negatively regulates cell fate determination genes such as let-60, the nematode RAS gene. Loss of let-60/RAS suppresses a let-7 mutation and the let-60/RAS 3'UTR contains multiple let-7 complementary sites, restricting reporter gene expression in a let-7 dependent manner. let-7 is conserved in humans, where it has also been linked to cancer. Specifically, human let-7 is poorly expressed or deleted in lung cancer, and over-expression of let-7 in lung cancer cells inhibits their growth, demonstrating a role for let-7 as a tumor suppressor in lung tissue. We have also shown that human let-7 is expressed in the developing mammalian lung and regulates the expression of important oncogenes implicated in lung cancer, including RAS. We found that human

HRAS, KRAS and NRAS 3'UTRs contain multiple let-7 complementary sites, and that let-7 regulates RAS expression through their 3'UTRs, suggesting conservation of gene interactions and a mechanism for let-7's involvement in cancer. We have identified numerous other miRNAs whose expression is altered in various cancers (oncomirs) and are identifying cancer gene targets of these miRNAs. We are focused on the role of let-7 and other oncomirs in regulating proto-oncogene expression during development and cancer, and on using miRNAs to suppress tumorigenesis.



Edward F. Srour
Indiana University
School of Medicine

Edward F. Srour, Ph.D., is a Professor of Medicine, Pediatrics, and Microbiology/Immunology at the Indiana University School of Medicine. Dr. Srour directs the Flow Cytometry Resource Facility of the Indiana University Cancer Center. The research program in the laboratory of Dr. Srour focuses on stem cell biology in general. In particular, three areas of interest are pursued, hematopoietic stem cells, adult stem cells, and cancer stem cells. Investigations concerning hematopoietic stem cells examine the relationship between cell cycle status and homing and engraftment of these cells as well as the impact of the hematopoietic microenvironment through ontogeny on these properties. Using a murine model, the laboratory investigates the differentiation and lineage specification potential of a group of common pluripotent adult stem cells detected in multiple tissues and the possible utilization of these cells in tissue repair and regenerative medicine. In collaboration with other investigators at Indiana University, Dr. Srour is working on identifying cancer stem cells in breast cancer and determining the relationship between putative cancer stem cells and metastasis.

Defining Subpopulations of Breast Cancer Stem and Progenitor Cells

It is believed that cancers arise from malignant transformations of normal stem/progenitor cells whereby these cells become responsible for tumor progression and possibly metastasis. Cancer stem cells (CSC) may therefore retain functional properties associated with normal stem cells including self-renewal and the generation of heterogeneous progeny. Whether CSC are responsible for metastasis is not clear. However, a genetic fingerprint of breast cancer cells with metastatic potential is beginning to appear. We have been investigating both in vitro and in vivo a number of breast cancer cell lines as well as primary breast cancer tumor samples to identify putative CSC populations and to examine the association between metastatic potential and the expression of "metastasis signature" genes. Cell lines with a substantial $CD44^+CD24^-$ sub-population were more invasive in an in vitro matrigel assay compared to cell lines without or with small numbers of $CD44^+CD24^-$ cells. Breast cancer cell lines with a $CD44^+CD24^-$ sub-population expressed higher levels of pro-invasive/metastasis genes (CXCR4, IL-11, CTGF, MMP-1, Osteopontin, IL-1α, IL-6, IL-8, and UPA). Interestingly, lines derived from the in vivo metastasis of a parent line with $CD44^+CD24^-$ cells expressed higher levels of pro-invasive genes and metastasized more efficiently than the parent cell line. However, not all cell lines with $CD44^+CD24^-$ cells were capable of pulmonary metastasis suggesting that the $CD44^+CD24^-$

phenotype, while associated with invasion, is not sufficient for the establishment of metastasis. Differential gene expression in MCF-10A-derived $CD44^+CD24^-$ and $CD44^+CD24^+$ cells revealed higher levels of stem cell genes and lower levels of pro-apoptotic genes in $CD44^+CD24^-$ cells. Additional phenotypic identification of MCF-10A CD44+CD24- cells revealed that in vitro cancer stem cell behavior such as matrigel invasion was most prominent among $CD44^+CD24^-CD326^-CD29^{hi}CD49f^{lo}$ cells. When a similar battery of antibodies were used to identify CSC in primary breast cancer cells, four sub-populations were distinctively identified; $CD44^+CD49f^-CD227^-CD326^-$, $CD44^+CD49f^-CD227^+CD326^+$, $CD44^+CD227^+CD49f^+CD326^+$, and $CD44^+CD49f^+CD326^+$. In matrigel assays, unlike normal cells from the breast, which form acinar structures with a hollow lumen, $CD44^+CD49f^-CD227^-CD326^-$ cells formed irregular colonies with no lumen. Cells from the other sub-populations did not form colonies. These studies present a strong link between $CD44^+CD24^-$ cancer cells and stem cell function, but not metastatic potential, and further identifies breast cancer stem cells as those expressing the phenotype $CD44^+CD24^-CD49f^-CD227^-CD326^-$.



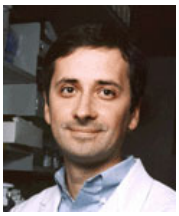
Sara Sukumar
Johns Hopkins University

Saraswati Sukumar, PhD is the Barbara B. Rubenstein Professor of Oncology and a Professor of Pathology at the Johns Hopkins University School of Medicine, Baltimore, MD. She co-directs the Breast Cancer Program, a division of the Sidney Kimmel Comprehensive Cancer Center. She heads a Department of Defense Center of Excellence at the Kimmel Center, and is a participant in the SPORE program. Her research interests lie in understanding the molecular events leading to the development of breast cancer, and translating these findings to the clinic. To this end, several of her laboratories' findings, such as in early detection using methylated genes as markers, and intraductal administration of drugs for treating and preventing breast cancer are in various stages of clinical development. Her studies in HOXA5 and HOXB7 have revealed many novel functions for these genes in mammary development and neoplasia.

HOXB7: At the Hub of Multiple Signaling Pathways Leading to Tamoxifen Resistance

Long term treatment of ER-positive breast cancer with tamoxifen often leads to the development of SERM resistance. In recent years, many signaling pathways that lead to tamoxifen resistance have been defined, but the upstream events controlling them are not known. Our work shows that overexpression of HOXB7 renders MCF-7 cells resistant to tamoxifen via mutually intensified tyrosine kinase receptor and ER signaling. MCF-7 cells treated with tamoxifen shows progressively increasing levels of HOXB7 expression over time, accompanied by coordinate increases in expression of EGFR/HER2 and ERα. It is likely that HOXB7 overexpression is a key event in the initiation and maintenance of tamoxifen resistance. Exposure of tamoxifen-resistant MCF-7 cells to Fulvestrant, a drug which promotes ER degradation, results in the normalization of overexpressed HOXB7 and EGFR/HER2 levels. Unlike tamoxifen, Fulvestrant remains effective in the treatment of xenografts of MCF-7-

HOXB7 cells. These studies suggest that HOXB7 is a master regulator orchestrating two major groups of target molecules that are critical in the development of SERM resistance. Functional antagonism of HOXB7 might be of great importance to circumvent the complexity of current breast cancer therapeutics.



Victor Velculescu
Johns Hopkins Univ.

Victor Velculescu, M.D., Ph.D., is internationally known for his genomic discoveries in human cancer. Dr. Velculescu developed SAGE (serial analysis of gene expression), a method for global gene expression profiling. This approach led him to coin the word "transcriptome" to describe comprehensive gene expression patterns that could now be analyzed in human cancer and other cells. Over the past decade, he has devised equally powerful technologies for analysis of the cancer genome. His group was the first to systematically analyze entire gene families for genetic mutations in human cancer. These efforts have recently identified frequent activating mutations in protein and lipid kinases and inactivating mutations in protein phosphatases not previously linked to neoplasia. In particular, he has found genetic alterations in phosphatidylinositol 3-kinase PIK3CA in a high fraction of colon, breast and other tumors, making this gene one of the most highly mutated oncogenes in human cancer. These findings provide novel targets for therapeutic intervention and open the door to individualized analysis and treatment of cancer.

Dr. Velculescu attended Stanford University where he graduated with Honors and Distinction in Biological Sciences. He obtained his M.D. and Ph.D. in Human Genetics and Molecular Biology at the Johns Hopkins University School of Medicine. He is currently Director of Cancer Genetics at The Ludwig Center and Associate Professor of Oncology at the Johns Hopkins University Kimmel Cancer Center.
Dr. Velculescu has received a variety of honors for his work in cancer genomics. These include the Grand Prize Winner of the Amersham/Pharmacia & Science Young Scientist Prize, The Pew Scholar Award, The Sir William Osler Young Investigator Award, and was one of Popular Science's "Brilliant 10" Young Scientists of 2004. He has given numerous invited lectures and has organized international conferences on topics related to his work.

Genomic Analyses of Human Cancer

It is widely accepted that human cancer is a genetic disease caused by accumulation of mutations in oncogenes and tumor suppressor genes. These tumor-specific mutations provide clues to the cellular processes underlying tumorigenesis and have proven useful for diagnostic and therapeutic purposes. To date, however, only a small fraction of the genes has been analyzed and the number and type of alterations responsible for the development of common tumor types are unknown. The determination of the human genome sequence coupled with improvements in sequencing and bioinformatic approaches have now made it possible, in principle, to examine the cancer cell genome in a comprehensive and unbiased manner. We have begun a systematic study of the cancer genome through examination of genes families involved in signal transduction. These efforts have recently identified frequent activating mutations in a number of different kinases and phosphatases not previously linked to human cancer. For example, we have found genetic

alterations in the PIK3CA gene encoding the p110 alpha phosphatidylinositol 3-kinase in ~30% of colon and breast cancers, providing a rational target for therapy in a large fraction of common malignancies. The results of these studies and their extension to genome-wide analyses of human cancer will be discussed.



Bert Vogelstein
Johns Hopkins University School of Medicine
Howard Hughes Medical Institute

Dr. Vogelstein graduated from the University of Pennsylvania in 1970 with a degree in mathematics. He obtained his medical degree at the Johns Hopkins University School of Medicine and performed his internship and residency in pediatrics at the Johns Hopkins Hospital. Following his clinical training, Dr. Vogelstein completed a post-doctoral fellowship at the National Cancer Institute. He returned to Johns Hopkins as an Assistant Professor in Oncology, and is now Clayton Professor of Oncology and Pathology and an Investigator of the Howard Hughes Medical Institute. In 2006, he was appointed Director of the Ludwig Center for Cancer Genetics & Therapeutics at the Johns Hopkins Kimmel Cancer Center.

Since 1978, Dr. Vogelstein has mentored many uniquely talented graduate students and post-doctoral fellows. Together, they have performed important studies on the pathogenesis of human cancers.

Translational Implications of Colorectal Cancer Genetics

Cancer is, in essence, a genetic disease. The implementation of this knowledge for improving patient care is the most challenging and important objective of cancer research. Using colorectal tumorigenesis as a paradigm, several fundamental ways in which the knowledge of cancer genetics can be used to reduce morbidity and mortality will be discussed. The first and most obvious approach is the development of new therapeutic agents that specifically target the genetic alterations present in cancers or the physiologic sequelae of these mutations. The strengths and obstacles inherent to these strategies will be reviewed. Other translational approaches that aim to prevent tumorigenesis or detect it at early stages represent particularly promising avenues for future research. These include (i) the development of genetic tests to predict who is at high risk for the development of specific cancer types, such as those of the colon, breast, or brain; (ii) the development of vaccines against infectious agents known to initiate specific cancer types, such as cervical and hepatic cancers; and (iii) the development of non-invasive tests for early cancer based on the detection of mutant genes or gene products *ex vivo*. These include tests for mutations in various bodily compartments as well as novel imaging modalities. The combination of gene-based diagnostics and genetically targeted therapeutics has the potential to significantly reduce morbidity and mortality from cancer in the foreseeable future.



Mark Wade
Salk Institute

Mark Wade is a postdoctoral research fellow in the Laboratory

of Professor Geoffrey Wahl at the Salk Institute for Biological Studies, La Jolla. Mark graduated from the University of Bath, UK with First Class Honors in Molecular Biology (1997). He received his PhD from Imperial College, London, UK in 2001 under the supervision of Dr. Martin Allday, where his project focused on the role of EBV in genotoxic responses in Burkitt lymphoma. During his graduate studies, Dr. Wade developed an interest in the relationship between oncogene activation and p53 function. This lead to recent work evaluating how p53 governs the switch between cell cycle arrest or apoptosis. Currently, Dr. Wade is investigating the molecular basis for hdmx-mediated attenuation of p53 function. He is involved in collaborations designed to understand the mechanism by which small molecules can re-activate p53 in human cancers, and aims to integrate these areas of research in his future career.

Hdm2 and Hdmx: Independent Targets for p53 Activation in Cancer?

The tumor suppressor p53 is wild type in many human cancers, providing a putative Achille's heel upon which to develop new therapeutic drugs. Current models indicate that wild type p53 activity in tumors can be suppressed by overexpression of hdm2, and the closely related hdmx protein. These proteins bind to p53 and control its stability and transcriptional activation function. A prevailing view is that hdm2 mainly controls p53 via its ubiquitin ligase activity, while hdmx, which lacks ubiquitin ligase function limits p53-dependent transactivation. A major research focus directed towards p53 reactivation in cancer has been the development of antagonists of the hdm2/p53 interaction. One such antagonist is the hdm2-binding compound, Nutlin, which stabilizes p53 and unleashes its transcriptional program to induce cell cycle arrest or apoptosis. Despite the homology between hdm2 and hdmx, here we present data demonstrating that Nutlin does not antagonize the hdmx/p53 interaction. We show that Nutlin can also induce degradation of hdmx, although this can be attenuated in some tumor cell lines. We and others have demonstrated that high levels of hdmx are associated with resistance to Nutlin, suggesting that hdmx antagonists may provide an additional chemotherapeutic strategy. Finally, we present data obtained using a novel compound that targets both the hdm2/p53 and hdmx/p53 interaction, and discuss future directions for research into small molecule inhibitors of p53.

Todd Waldman
Georgetown University

Todd Waldman, M.D., Ph.D. is an Associate Professor of Oncology at the Lombardi Cancer Center, Georgetown University School of Medicine in Washington, D.C. He received his M.D., Ph.D. from Johns Hopkins University in 1997, where he developed techniques in human somatic cell gene targeting and employed them to study the p53 pathway. In 1998 he was appointed Assistant Professor of Oncology at Georgetown, and was promoted to Associate Professor with tenure in 2005. As an independent investigator, Dr. Waldman's research has focused on further expanding the use of human somatic cell gene targeting to study various oncogene and tumor suppressor pathways and create reagents for target-based drug discovery. His current research interests focus on studying the phenotypic consequences of PTEN loss in human cancer cells. In addition to his research, Dr. Waldman serves as an associate editor of Cancer Research and Cancer Biology and Therapy, and serves as a member of the NIH Molecular Oncogenesis (MONC) study section.

PTEN Phenotypes in Human Cancer Cells

Mutations in the PTEN tumor suppressor gene are found in a wide range of common human cancers. PTEN is a PIP3 lipid phosphatase; therefore, loss of PTEN leads to activation of the PI3K signaling pathway. Dr. Waldman lab has recently employed human somatic cell gene targeting to study the function of PTEN in human cells. In his talk, Dr. Waldman will present data implicating PTEN in control of the cell cycle and cell size, and in response to radiotherapy.

Richard F. Wooster
GlaxoSmithKline

Dr. Richard Wooster studied at the Department of Biochemistry at Dundee University, Scotland. After this he moved to the Institute of Cancer Research in London where his work focused on the discovery of cancer susceptibility genes, particularly for breast cancer. This lead to the localisation of the BRCA2 susceptibility gene to chromosome 13 and then the cloning of the same gene.
During the cloning of BRCA2, Richard, spent time at the Sanger Centre, Cambridge, UK, and he moved to this institute to lead the characterisation of chromosome 1 for the human genome project. The availability of the human genome sequence empowered cancer research and Richard was a co-founder and leader of the Cancer Genome Project at the Sanger Institute, Cambridge, UK. This project commenced in 2000 to harness the power of DNA sequencing to characterise the cancer genome and identify genes which are mutated in cancer. This work lead to the identification of somatic mutations in many genes, some of which are unlikely to be disease causing but others such as BRAF and ErbB2 are clearly involved in the development of cancer. As part of this Dr. Wooster was the founder of the COSMIC database of somatic mutations found in cancer and the web site that gives access to this data.

Currently, Dr. Wooster is the Director of Cancer Genetics at GlaxoSmithKline. In this role he is identifying predictive biomarkers to identify the patients who are more likely to respond to therapeutic treatments being developed by GSK.



Gen Sheng Wu
Karmanos Cancer Institute

Dr. Gen Sheng Wu received his Ph.D. in Molecular Immunology at the Peking Union Medical College/Chinese Academy of Medical Sciences, Beijing, China, and was trained as a postdoctoral fellow in Molecular Oncology at the Howard Hughes Medical Institute at the University of Pennsylvania, Philadelphia, PA. He is currently an Associate Professor in the Department of Pathology and member of Barbara Ann Karmanos Cancer Institute at the Wayne State University School of Medicine, Detroit, MI. His current research interests include the areas of tumor suppressor genes, signaling transduction, the mechanisms of chemoresistance, and targeted cancer therapies.

Mitogen-Activated Protein Kinase Phosphatase-1 Signaling in Drug-Resistance

MKP-1 is a member of the mitogen-activated protein kinase (MAPK) phosphatase family that negatively regulates MAPK signals including ERK, p38 and JNK. Although MKP-1 has been shown to over-express in human cancer and has been implicated in cell survival in response to stressful stimuli, but its regulation in cancer cells is not

fully understood. We explored the mechanism by which MKP-1 is regulated in human cancer cells and identified MKP-1 as a potential target of p53. We showed that p53 regulates MKP-1 expression through two p53 responsive elements of the MKP-1 gene. Importantly, overexpression of MKP-1 prevented arrested cells from re-entering into the cell cycle, while inhibition of phosphatase activity by a chemical inhibitor impaired p53-mediated growth arrest in arrested cells in response to growth factor stimuli, suggesting that MKP-1 plays an important role in regulating the re-entry of arrested cells into the cell cycle in response to mitogens.

In addition, because induction of JNK and p38 is implicated in cisplatin-induced cell death and because MKP-1 can inhibit the activities of these two MAPK kinases, we investigated the role of MKP-1 in cisplatin-induced cell death. We found that cisplatin induces MKP-1 expression in both lung and ovarian cancer cell lines. Importantly, knockdown of MKP-1 sensitized lung cancer cells to cisplatin-induced cell death while overexpression of MKP-1 protected lung cancer cells from cisplatin-induced death. Furthermore, using primary murine embryonic fibroblasts (MEF) from MKP-1 knockout mice, we showed that induction of MKP-1 by cisplatin correlates with inactivation of JNK, but not ERK and p38. In addition, apoptosis induced by cisplatin was significant in MKP-$1^{-/-}$ MEFs while such change was minimal in MKP-$1^{+/+}$ MEFs. More importantly, blocking JNK but not ERK and p38 activities inhibited cell death induced by cisplatin. Thus, our results establish a critical role of JNK in cisplatin-induced apoptosis and suggest that MKP-1 is a therapeutic target for cancer therapy.

Submitted Abstracts

Pro-metastatic Signaling by c-Met through RAC-1 and Reactive Oxygen Species (ROS)

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Overexpression of the c-Met/hepatocyte growth factor receptor (HGF-R) proto-oncogene and abnormal generation of intracellular oxygen species (reactive oxygen species (ROS)) have been linked, by independent lines of evidence, to cell transformation and to malignant growth. By comparing two subpopulations of the B16 mouse melanoma (B16-F0 and B16-F10) endowed with different lung metastasis capacities (low and high, respectively) we found that both the expression/phosphorylation of c-Met and the steady-state levels of ROS positively correlated with metastatic growth. ShRNA-mediated downregulation of c-Met in F10 cells led to a parallel decrease in the generation of oxygen species and in metastatic capacity, suggesting that oxidants may mediate the pro-metastatic activity of the HGF receptor. c-Met activation by a ligand elicits the formation of oxidant species through the oxidase-coupled small GTPase Rac-1, a relevant downstream target of the HGF-R. Moreover, cell treatment with the catalytic ROS scavengers EUK-134 and EUK-189 attenuates Met signaling to ERKs and inhibits the anchorage-independent growth of F10 cells, consistent with a critical role for oxygen species in HGF signaling and in aggressive cell behavior. Finally, genetic manipulation of the Rac-ROS cascade at different levels demonstrated its crucial role in the pro-metastatic activity of c-Met in vivo. Thus, we have outlined a novel cascade triggered by c-Met and mediated by ROS, linked to metastasis and potentially targetable by new antimetastatic, redox-based therapies.

Functional Interactions of p57KIP2 and p53 during Immortalization of Human Mammary Epithelial Cells

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Immortality is a prerequisite for the accumulation of multiple malignant changes in a single cell lineage during human breast carcinogenesis. We use cultured human mammary epithelial cells (HMEC) to study changes contributing to immortalization. The immortal HMEC line 184A1 was obtained following treatment with a carcinogen, benzo(a)pyrene. This cell line expressed wild type p53, and initially displayed very low telomerase activity. When the cells attained mean telomere lengths of 3 kb or less, they reproducibly entered a period of slow, heterogeneous growth termed conversion, which was accompanied by accumulation of the cyclin-dependent kinase inhibitor p57KIP2. Up-regulation

of p57KIP2 during conversion was due to telomeric dysfunction, and could be blocked by the addition of exogenous telomerase. As conversion of 184A1 progressed, endogenous telomerase repression was alleviated, and p57KIP2 was down-regulated. To further investigate the role of p57KIP2 in HMEC immortalization, we used shRNA to inhibit its expression in pre-conversion 184A1. Surprisingly, despite the established function of p57KIP2 as a cell cycle inhibitor, decreased expression of this protein induced rapid growth arrest in pre-conversion HMEC, and prevented their transformation to full immortality. We found that the growth arrest induced by p57KIP2-shRNA in pre-conversion cells correlated with increased levels of p53-Ser15 phosphorylation and up-regulation of p21CIP1. When p53 was inhibited in 184A1 cells prior to the onset of conversion, telomerase was up-regulated, and p57KIP2 was down-regulated. We are further exploring functional interactions linking p53 and p57KIP2, as well as causes and consequences of the growth arrest induced by down-regulation of p57KIP2 in pre-conversion cells. By examining the mechanisms inhibiting and promoting HMEC immortalization, we expect to gain further understanding of rate-limiting processes taking place during early breast carcinogenesis.

Follicle Stimulating Hormone Receptor (FSHR) Antagonist and Epithelial Ovarian Cancer (EOC)

Bose, Chinmoy K Research Fellow, Medical, College, Kolkata & Specialist Medical Officer, North Maternity Home, Kolkata

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Introduction: Follicle stimulating hormone receptor (FSHR) is G-protein coupled receptor present in granulosa cell of ovary is a unique transmembrane molecule pivotal in ovulation process. Its agonist and antagonists has remained the subject of interest to the reproductive biologists. It seems worthwhile to see what new research on FSHR is and whether they will be of any help in epithelial ovarian cancer.
Methods: Pubmed and Medline search was made from January 2006 to April 2006 to find out current development of FSHR antagonist research and role of FSHR in epithelial ovarian cancer
Results: Effort to develop FSHR antagonist was mainly aimed at peptide antibody development. Breakthrough discovery of nonpeptide FSHR antagonist molecules, like suramin, compound 1 and compound 10 are discussed in the context of nonsteroidal contraceptive for male and female. FSHR was found to have a distinguished role in such cancer
Conclusion: Author proposes the trial of these molecules as novel anticancer agents in epithelial ovarian cancer.

Modeling of Tumor Response to Treatment

Dartmouth Medical School

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While mathematical modeling of untreated tumor progression is a well developed area (Gompertz curve), little or nothing is available for modeling growth and regrowth of treated tumors as a parsimonious function of the treatment dose. We have developed a novel theory for tumor regrowth, similarly to Gompertz curve for untreated tumors. This methodology is used for quantitative treatment comparison, finding the optimal treatment regimen and modeling therapeutic tumor resistance. All aspects of tumor regrowth theory are discussed, from statistical estimation to interpretation. Our approach is illustrated with data on chemotherapy and photodynamic treatment of tumors in animals.

Wnt5a Regulates Melanoma Antigen Recognized by T-Cells 1 (MART-1), a Predominant Antigen in Melanoma Cells

Samudra K. Dissanayake1, Kyle, J. Hewitt1, Devin T. Rosenthal1 Sherry Yang1, Michael Wade1, Poloko Leotlela1, Dennis D. Taub1, Brian J. Nickoloff2, Jeffrey M Trent3, Michael Bittner3 and Ashani T. Weeraratna1.
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We have demonstrated that Wnt5a expression increases melanoma cell motility via activation of Protein Kinase C (PKC). Microarray analysis of Wnt5a-overexpressing cells and cells treated with RNAi against Wnt5a showed an inverse relationship between Wnt5a and MART-1, an antigen important for the immune surveillance of melanoma. To determine if PKC activation could affect the expression of MART-1, melanoma cells lacking Wnt5a were treated with the PKC inducer phorbol myristic acid (PMA), which resulted in increases in Wnt5a, and decreases in MART-1 expression. Cells treated with recombinant Wnt5a also resulted in decreases in MART-1 expression, and increases in phospho-PKC. Similarly, inhibition of PKC activity corresponded to increases in MART1, and RNAi knockdown of Wnt5a also caused an increase in MART-1 levels. STAT3 is a transcription factor known to inhibit the expression of MART1 and its activation is dependent upon phosphorylation. PKC activator/ inhibitor studies indicate that STAT3 phosphorylation can be increased by PKC, and that phosphorylation of STAT3 correlates to decreases in MART1. Additionally, Wnt5a transfectants exhibit a translocation of pSTAT3 from the cytosol to the nucleus indicating activation of STAT3 by Wnt5a, while the reverse was observed for Wnt5a-positive cells transfected with siRNA against Wnt5a. In a functional assay designed to assess the consequences of Wnt5a reduction of MART-1 levels, melanoma cells expressing MART-1 were incubated with MART-1 specific

cytotoxic T-lymphocytes (CTLs). MART1 positve melanoma cells were able to activate CTLs, but these same melanoma cells, upon exposure to recombinant Wnt5a could not, presumably due to the down regulation of MART-1. RNA from human melanoma biopsies analyzed for Wnt5a by real-time PCR show an inverse relationship between Wnt5a levels and patient survival time, suggesting that cells with higher MART-1 have a better outcome. Collectively, our data suggest that Wnt5a, via PKC, results in the phosphorylation of STAT-3 and a subsequent down-regulation MART1 thereby rendering melanoma cells able to escape immune detection.

Acquired Cisplatin Resistance by BRCA1-IRIS Overexpression in Human Ovarian Epithelial Cells

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Overexpression of BRCA1-IRIS in human ovarian surface epithelial cells (HOSE) induced the expression of AKT1 and 2 as well as the anti-apoptotic protein Survivin. It has been shown earlier that AKT1 and AKT2 both activate Survivin expression in ovarian cancer cell lines. Whether IRIS-dependent Survivin overexpression is dependent on activation of AK1 and/or 2 expressions was tested. In HOSE cells overexpressing BRCA1-IRIS, treatment with PI3`K inhibitor (LY294002), dominant negative AKT or AKT siRNA only partially reduced IRIS-dependent Survivin overexpression, suggesting that IRIS activates Survivin in an AKT-dependent as well as independent manner. Cisplatin is a widely used chemotherapy drug to treat ovarian cancer as well as other type of cancers. Treatment of HOSE cells with Cisplatin showed an interesting pattern. While at concentration >50µM Cisplatin reduced IRIS, AKTs and Survivin expression, and induced dramatic increase in cell death. At concentrations 30-50µM, BRCA1-IRIS and not AKT or Survivin level was reduced, yet cells still showed increase in cell death, suggesting that BRAC1-IRIS induces ovarian cell survival is both Survivin-dependent as well as independent. Importantly, at concentration <30µM, Cisplatin instead increased the expression of all three proteins. In fact at these concentrations no increase in cell death was measured, raising the interesting possibility that at lower concentrations Cisplatin actually protects ovarian cells by increasing the levels of all three proteins. Furthermore, only HOSE cells depleted from IRIS (not AKT or Survivin) were prone to death when treated with Cisplatin even at low concentrations (e.g., 35µM). Taken together, these data beside bringing the question whether drug-resistance and/or recurrence could in fact be a side effect of the drug used itself, it also suggest that reduction in BRCA1-IRIS expression/activity might sensitize ovarian cancer cells to chemotherapeutical drugs, such as Cisplatin.

The Anticancer Effect of Morus Alba Leaves Extract In Vivo (Solid Ehrlich Carcinoma) And In Vitro (Eac-Cells)

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The chloroform extract (lipid soluble fraction) of Mores alba is the most effective in the in vitro study as 0.5 mg produced 95% dead cells. The MTD (maximum tolerated dose) is 0.48 g/kg b.w). The.LD12.5 was used for the treatment of solid Ehrlich carcinoma bearing mice for 10 weeks every other day, starting from the 9th day post EAC-cells implantation, in two groups: 1 hr and 9 days post implantation. Results showed an increase of 121.6%, and 185.4 % in the ILS (life span prolongation) respectively. This indicated the efficacy of the used extract as a curative agent more than a protective one. Also, this mode of treatment ameliorated the levels of serum total protein and its fractions, total bilirubin and its fractions, ALT, AST and G-6-PDH activities, and liver total lipids. The extract has no detrimental hepatotoxicity indicated by the normalized liver 5'Nase activity. As well, the treated group mean b.w. was only 1/3rd the untreated group after 37 day. It is note worthy to mention that the extract has a remarkable antiglycemic effect as the glucose blood level decreased by 31% after 8 w. in the normal treated group; also liver glycogen was depleted in both treated groups, which may be a contributing factor in limiting tumor growth. The histopathology study confirms the biochemical one. Key words: Morus Alba, EAC-cells, liver enzymes, ILS.

Identification of Invasive and Metastatic Candidate biomarkers Associated with Nasopharyngeal Carcinoma Based on Microarray and Literature Profiles of Pubmed

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Invasion and metastasis of cancer cells are both of the most important factors that influence the prognosis of patients with nasopharyngeal carcinoma (NPC). Previous studies linking the molecular mechanism of NPC invasion and metastasis are seldom performed. To better understand molecular basis of NPC invasion and metastasis, we screened candidate invasion and metastasis biomarkers associated with NPC by using gene expression profiles between selected NPC tissues and proximity normal nasopharynx (NP) tissues and online MILANO software analysis. Microarray data from pooled NPC cells including 5-8F, 6-10B and CNE2 vs NPs help us adjunctively

analyze the roles of part candidate genes. The results displayed that candidate 51 upregulated and 23 downregulated genes are associated with invasive and metastatic characteristics shown in human malignancies. However, nearly all of them have never been reported in NPC-associated literatures. Among these genes, most of them including IGFBP, MIF, ECGF1, P8, ECGF1, MMP12, PTTG1, MMP1, HDGF, PTGS2, PLAU, P311, BPAG1, NSEP1, TGFBR2, CTGF and MGP etc exert their actions in invasion and metastasis of cancer cells corresponding to the reports in Pubmed literatures. Nevertheless, other some genes including NK4, GPC3, NME2 and ARF6 etc display possibly negative regulation inverse with the reported roles of Pubmed articles. Some special genes such as known anti-metastasis gene NME1 showed contradictory results in tumor invasion and metastasis. Individual gene, EGR1, showed inverse results compared to previous studies reported in NPC. Loss of EGR1 expression may not only contribute to the carcinogenesis, but also promote NPC invasion and metastasis. These screened genes, after GOstat analysis, are likely to associate with signal transduction, cell adhesion, cell communication, cell motility, cell proliferation, regulation of biological process and apoptosis etc in the process of NPC invasion and metastasis.

The Roles of a Splicing Variant of FANCL

Jun Zhang, Xianshu Wang, Chia-Ju Lin, Wangou Liu and Peiwen Fei

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The roles of a splicing variant of FANCL Jun Zhang, Xianshu Wang, Chia-Ju Lin, Wangou Liu and Peiwen Fei Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine Fanconi Anemia results from mutations in a group of genes, whose products, including BRCA2 and BACH1/BRIP1, are known to function in one common pathway, named the FA-BRCA pathway to guard genome integrity. DNA synthesis or DNA damage, especially triggered by DNA crosslinking agents, such as Cisplatin and mitomycin C (MMC), can activate this pathway, within which a multi-protein complex, including eight of known FA proteins (FANCA/B/C/E/F/G/L/M) acts as a ubiquitin-ligase E3 to monoubiquitinate FANCD2, and therefore activate this pathway. The monoubiquitinated FANCD2 then partners with BRCA2/FANCD1, BRIP1/BACH1/FANCJ, and others to form the second protein complex for DNA damage repair. The extremely high incidence of cancer formation in FA patients reveals the essentialness of this pathway in tumor suppression. However, how this pathway is involved in non-FA cancers is not well understood. Examination of 10 randomly selected tumor cell lines revealed that an impaired FA-BRCA pathway is harbored in a lung tumor cell line. Studies on searching the cause(s) of this impaired pathway identified that a splicing variant of FANCL, the catalytic subunit of the ubiquitin ligase E3 FA complex, is expressed in this cell line. To assess the possible roles of this variant in tumor

formation, we started to examine its expression in 20 prostate tumor samples along with 20 matched normal tissues as controls accordingly, and found that this variant is expressed in all tested samples, but only highly expressed in 4 or 5 of 20 tumor samples. To reveal the potential functions of this variant, we overexpressed this variant in PA1 cells and found these transfected cells following exposure to cisplatin, contained a compromised FANCD2 monoubiquitination and committed relatively higher cisplatin sensit.

The Effect of p21 Gene Expression in Human Hepatocelluar Carcinoma Cell Line HepG2 Proliferation by the Venom of the Spider Macrothele Raveni

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To study the effects on the proliferation of human hepatocelluar carcinoma cell line HepG2 by the venom of the spider Macrothele raveni and the molecular mechanism of the action. Using the MTT assay of the inhibition of HepG2 cells, the proliferation was examined; the cell cycle change in HepG2 cells treated by the spider venom was investigated by flow cytometry. The effects on expression of p21 was studied by reverse transcriptase PCR; In this study MTT test showed that the proliferation of BEL cells in vitro was inhibited by spider venom ($P<0.05$) in a concentration-dependent manner. Flow cytometric analysis showed that the spider venom caused selective cell cycle arrest of HepG2 cells. Expression of p21 mRNA was obvious up-regulated by the spider venom for 72hr of HepG2 cells. The venom of the spider Macrothele raven inhibited the proliferation of human hepatocelluar carcinoma cell line HepG2. The possible mechanism was to activate the lower expression of p21 gene and to cause selective cell cycle arrest of HepG2 cells, then to induce the HepG2 to apoptosis.

Spider Venom—A Novel Material with Anti-Tumor Activity

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We are first to report on the antineoplastic activity of the venom of the spider, Macrothele raveni (Araneae, Hexathelidae) on human breast carcinoma cell line MCF-7. Cell death is a tightly controlled process and may occur by apoptotic or non-apoptotic means known as necrosis. Apoptosis is the regulated destruction of a cell and may be triggered by either external or internal signals. The characteristic morphological changes of apoptosis were observed by Acridine Orange/Ethidium Bromide (AO/EB) staining, scanning electron microscopy (SEM) and transmission electron microscopic (TEM). The growth-inhibitory effects of these agents were divided into dose- and time-dependent manners by [3H]-methyl thymidine incorporation assay ([3H]-TdR).

Cytotoxicity of MCF-7 cell by spider venom at concentrations of 10, 20, 40μg/mL was determined by lactate dehydrogenase (LDH) release assay. Spider venom was investigated to induce the apoptosis and necrosis of MCF-7 cells at 10, 20, 40μg/mL concentration by flow cytometry (FCM). MCF-7 cells were accumulated on G2/M phase by spider venom after 24h. Furthermore, Western blotting analysis indicated one of the pharmacological mechanisms of spider venom is to activate the expression of P21. In vivo study of the inhibition of the size of tumors of nude mice by the spider venom (1.6,1.8,2.0μg/g mice) have shown that tumor size was significantly decreased from controls by 21 days of treatment and at all points of analysis there after for 7 week ($P<0.01$).

Effect of Solanine on the Contents of Caspase-3 and Bcl-2 in Hepg2

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Commonly called "nightshade", Solanum nigrum L is an annual plant belonging to the Solanaceae family. It is found throughout China, but is more abundant in the Northeast and North China regions. Its main contents are alkaloids, but it also contains vitamins and many trace elements. There have been reports of the plant being used in folk medicine to treat tumors with good effect. In earlier experiment, we found that solanine can significantly prolong the survival time of H22 tumor-bearing mice. In the present experiment we observed the effect of solanine on the contents of caspase-3 and bcl-2 in HepG2, and to explicate the mechanism by which solanine induces the apoptosis of tumor cells. Laser confocal scanning microscopy (LCSM) and Western blot were used to measure the contents of caspase-3 and bcl-2, and LCSM was used to determine their locations in the cell. The results demonstrated Solanine could remarkably increase the content of caspase-3, at the same time decrease the content of bcl-2 in HepG2, both had close correlation with dosage of Solanine. Both caspase-3 and bcl-2 were unevenly distributed in the cytoplasm, but were not found in the nucleus. Solanine did not affect their distribution. Finally we concluded that by suppressing the activity of Bcl-2, solanine could activate the caspase-3 protease family and thus induced cell apoptosis. Keywords Solanine; Caspase-3; Bcl-2; Anti-tumor mechanism Acknowledgement This work is supported by the National Science Foundation of China(No.30400591,30300284), the National Science Foundation of Heilongjiang province(No.D2004-13), Science Foundation of Harbin for Youth(No.2004AFQXJ035) and natural science foundation of Harbin University of commerce for postgraduates(YJSCX2005-134HLJ).

Identification of Novel Cancer Drugs Using Phage Display

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We have utilized a display approach to identify novel lead candidates for oncology indications. Using high diversity peptide libraries, we have isolated pharmacologically active peptides (called HotSpot Pharmacophores or HPs) against both validated targets (i.e., tyrosine kinases) and novel targets. We have identified HP antagonists against several key tyrosine kinases including EGFR, FGFR1-4, PDGFR, c-Met and IGF-1R. In the case of IGF-1R, several HPs have been shown to block receptor phosphorylation and inhibit cancer cell proliferation in an IGF-1 dependent manner. In addition, two antagonists have been tested in animal models of human pancreatic cancer and significantly inhibit tumor growth. In addition, we have developed a technology platform which allows rapid identification of cancer-specific HPs and we have generated one which inhibits the growth of human prostate cancer xenografts in vivo. We are presently in the process of moving one of these candidates into the development stage as a prelude to Phase I clinical trials.

Identification and Characterization of Invasive and Drug Resistant Breast Cancer Cells

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Metastasis and drug resistance are major hurdles in curative cancer treatment. More attention is needed in decoding these aspects of cancer in order to develop more effective therapies. Recently we have discovered that the invasive breast cancer cells in rodent models are resistant to chemotherapeutic drugs. Identification of molecular markers for both invasive and drug resistant cells from within the primary tumor would open a new vista in cancer molecular diagnosis. These would also potentially identify new pathways for therapeutic intervention which will be a useful adjunct to conventional therapies, interfering with tumor progression at several pivotal points. We have developed an in vivo invasion assay, which provides an opportunity to collect primary tumor cells that are actively in the process of invasion. The in vivo invasion assay has been combined with array-based gene expression analyses to investigate the gene expression patterns of carcinoma cells in primary mammary tumors during invasion. The expression of genes involved in cell division and survival, and cell motility were most dramatically changed in invasive cells indicating a population that is neither dividing nor apoptotic but intensely motile. We have performed biological assays to show in two different rodent

models that the invasive carcinoma cells are hypoproliferative and drug resistant even before any therapeutic intervention is done on them. This invasion signature provides a general resource of possible targets for future anti-invasion and drug resistance therapy. We have also been successful in isolating the cancer cells at different steps of the metastatic process and studied their gene expression pattern and have identified a set of genes whose expression is altered transiently due to the tumor microenvironment.

Hif-1α Independent Down-regulation of VEGF Expression in HL60 Cells after In Vitro Exposure to ATRA

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Although the biologic effects of ATRA so far are well characterized, the molecular mechanisms regulating these processes are largely unknown. The expression of VEGF has been widely demonstrated in various human malignant tumours including haematological malignancies. Some results demonstrated that high expression of VEGF can promote the proliferation and colony formation and inhibit the apoptosis of leukemic cells, which promotes the progression of hematopoietic tumors not only by stimulating vascular endothelial growth. But the molecular mechanism of modulation of VEGF secretion and expression by ATRA in leukemia cells, for example HL60 cells, has not been elucidated clearly. The study was aimed to investigate the anti-leukemic mechanism of ATRA in respect of vascular endothelial growth factor (VEGF) production and which pathway contributed to the molecular mechanism of VEGF expression modulation. Semi-quantitative RT-PCR and ELISA were used to detect the expression of VEGF mRNA and secretion of VEGF protein in HL-60 cell lines treated by ATRA respectively. The results showed that the expression of VEGF mRNA and secretion of VEGF protein were found in HL-60 cells, which could be down regulated by exposure to ATRA for 48 and 72 hours. Level of Metalloproteinase MMP9 but not MMP2 in the culture supernatant of HL60 cells was correlated with VEGF secretion, which was demonstrated by the special inhibition of MMP inhibitor batimastat (BB-94) or ATRA treatment. It indicated that MMP9 played a role in the release of biologically active VEGF. ATRA exerts its anti-leukemia effects by reduction of the expression of VEGF mRNA and VEGF protein secretion. The VEGF mRNA or c-Myc mRNA expression was down-regulated, otherwise, HIF-1α mRNA was up-regulated in HL-60 cells after exposure to ATRA. The antisense c-Myc could block the VEGF mRNA expression, which indicated that the ATRA could reduce the VEGF mRNA expression by down-regulation of c-Myc.

The Molecular Mechanism of Trichostatin A Facilitate Arsenic Trioxide Induce HL-60 Cells Differentiation

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Objective: In order to study if Trichostatin A facilitate Arsenic Trioxide induce HL-60 cells differentiation and explore their underlying mechanism.
Methods: the alteration in the cell proliferation was determined by MTT experiment; NBT reduction assay and inspect differentiation antigen on cellular surface was used to detect the differentiation condition of cells; Determinate and analysis cell cycle by FCM; The expressions of mRNA of P21 and Cyclin D3 in HL-60 cells after exposure to TSA and (or) As2O3 were assayed by semi-quantitative RT-PCR.
Results: It was found that TSA combine As2O3 could inhibit proliferation and induce differentiation obviously than single use As2O3, and up-regulate the mRNA expression of P21 but down-regulate the expression of Cyclin D3.
Conclusions: TSA has synergistic effect with As2O3 in induce HL-60 cells differentiation and it's associated with the change of expression of P21 and Cyclin D3. Keywords: Trichostatin A; Arsenic Trioxide; HL-60 cell; induce differentiation; synergistic effect.

Involvement of Caspase-3, Iron Regulatory Protein-1 and Nitric Oxide Synthase in the Antimetabolite Chemotherapy Mediated Response in Nitric Oxide Sensitive versus Resistant Malignancies

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Nitric oxide (NO) could be pro- or anti-apoptotic depending on cell type and caspase-3 activity. We aimed at studying the effect of antimetabolite chemotherapy on the enzymatic activity of nitric oxide synthase (NOS), caspase-3 and iron regulatory protein-1 (IRP-1) in 20 newly diagnosed acute myeloid leukemia (AML) patients and 10 hepatitis C virus infected hepatocellular carcinoma (HCC) patients since they have varied sensitivity to NO. AML patients received 7+3 regimen (combination of cytosine arabinoside (ara-C) and doxorubicin (DOX) and HCC patients received 5-fluorouracil (5-FU) 500 mg/m2 for 5 days every 3 weeks. All patients were followed up for 6 months to assess their response to therapy. The effect of adding S-nitroso-N-acetyl-penicillamine (SNAP), an NO donor, to chemotherapy on malignant cell survival of mononuclear cells (MNC) from AML patients (NO sensitive) and hepatocytes (NO resistant) from HCC patients was studied in vitro. Ten AML patients in remission and 10 patients having chronic hepatitis C (CHC) of matched age and sex served as controls for AML and HCC patients

respectively. Results showed a significant decrease in plasma NOS activity in AML than HCC cases. Also, the MNC and hepatocytes were significantly different in cell aconitase activity, iron content and caspase-3 activity. Significantly higher NOS activity at diagnosis in chemosensitive AML patients compared to chemoresistant patients was also shown. Better chemotherapy response has been associated with significant decline in plasma NOS, MNC aconitase activity and iron content and rise of cellular caspase-3 activity compared to their levels at diagnosis. The HCC cells had significantly higher cellular iron content than chronic hepatitis –C infected cells. The former cells were resistant to 5-FU in vivo and in vitro. In vitro 6-h incubation of MNC with ara-C/DOX/SNAP combination was associated with lower percent cell survival, higher caspase-3 activity than cells incubated with ara-C or SNAP/ara-C. Conversely, HCC was insignificantly affected by incubation with 5-FU alone or with SNAP. The cytosolic aconitase activity was significantly inhibited in MNC and HCC following incubation with SNAP alone or with chemotherapy compared to corresponding cells in medium. A decline in intracellular iron was a feature that accompanied sensitivity to chemotherapy in AML cells in vivo and in vitro. This denotes that the interplay between NO level, caspase-3 activity and IRP-1 level largely determines antimetabolite chemosensitivity in different cells. NO donor may have a role in increasing chemosensitivity in AML while its value appears limited in HCC.

Brain-Derived Neurotrophic Factor Triggers Signaling Cascades Mediating Growth, Migration, and VEGF Secretion in Multiple Myeloma Cells

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Multiple myeloma (MM) is an incurable form of cancer characterized by accumulation of malignant plasma cells in the bone marrow. Recently, it has been demonstrated that brain derived neurotrophic factor (BDNF) is crucially involved in the angiogenesis in MM. In this study, we examined the biological effects of BDNF on MM cells and the signaling pathways involved. We observed that BDNF promotes proliferation and migration of both MM cell lines and primary MM cells in a dose-dependent manner, as a consequence of TrkB activation. VEGF is also induced by BDNF in MM cells. Stimulation of MM cells with BDNF led to the activation of the phosphatidylinositol 3-kinase/Akt and the MEK-extracellular signal-regulated protein kinase (ERK) pathways. Using specific signal-transduction inhibitors, we demonstrated that MEK is required for BDNF-induced proliferation, whereas activation of PI3K is required for BDNF-stimulated migration and VEGF production. In conclusion, BDNF

affects different cell signaling pathways mediating growth, migration, and VEGF secretion in MM cells. Our observations provided the framework for novel therapeutic strategies targeting BDNF signaling cascades in MM. Keywords: multiple myeloma; brain-derived neurotrophic factor; proliferation; migration; signal transduction.

First Comprehensive Report of High Frequency of Pre-Existing Bcr-Abl Gene Atp-Binding Domain Mutations Associated With Natural Gleevec Resistance in Chronic Myeloid Leukemia (Cml) Patients

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Imatinib monotherapy is gold standard for CML patients. It is the first targeted therapy for cancers. Although very successful at all CML stages, imatinib resistance (acquired and natural) has been reported in most of the CML patients, making Imatinib resistance as one of the most emerging issues of pharmacogenomics. Point mutations in ATP-binding domain of BCR-ABL fusion gene has been detected in resistant patients which disturb the binding of Imatinib to its target bcr-abl oncoprotein, leading to resistance. Studies show that in some cases, mutations pre-exist the therapy and lead to natural Imatinib resistance. Detection of pre-existing mutations can help in deciding the potential responders and poor responders of the treatment and can help in adjusting the treatment accordingly, thus leading to personalized medication. In this study, pre-existing BCR-ABL ATP domain mutations were detected in CML patients who developed Imatinib resistance, later on i.e. on initiation of the therapy. ASO-PCR was employed to detect point mutations in CML patients prior to Imatinib therapy. Upon initiation of therapy, follow-up studies were carried out to know the Imatinib resistance. A total 52 patients were studied for three mutations. Mixed mutations were detected in 18 (36%), mutation T1052 in 16 (31%), mutation T932C in 8 (15%) and mutation C944T in 7 (11%) patients. All patients showed resistance to Imatinib after therapy initiation. This research report shows that mutations in BCR-ABL ATP-binding domain exist prior to therapy and mutant clones probably proliferate after therapy initiation, leading to natural Imatinib resistance. These findings of all great clinical importance in managing resistance to imatinib and other such drugs and open ways for personalized medication. It will further lead towards understanding molecular mechanisms of resistance to molecularly targeted cancer therapies and to develop the strategies not only to overcome this resistance but also to design more effective drugs.

Study on Effects of Two Kinds of Cactus Polysaccharides on the Protein Contents and the Lipid Fluidity of Erythrocyte Membrane in S180 Mice

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Objectives: To study the effects of two kinds of cactus polysaccharides on band 3 protein content, cross-linking protein content, and the lipid fluidity of erythrocyte membrane in S180 mice.
Methods: Membrane protein content is analyzed by SDS-PAGE, and lipid fluidity of the membrane is measured by the Skinitzky method.
Results: The two kinds of cactus polysaccharide increase the content of band 3 protein, decrease the content of cross-linking protein, and increase lipid fluidity. For medical cactus polysaccharide, the effect of the medium dosage group is significant ($P<0.01$); but for edible cactus polysaccharide, it is the high dosage group that has a significant effect ($P<0.01$).
Conclusions: By improving the functioning of erythrocyte membrane of tumor-bearing mice, the two kinds of cactus polysaccharides enhance their immunological function, and this may be one of the anti-tumor mechanisms of cactus polysaccharides.
Keywords: cactus; polysaccharide; band 3 protein; cross-linking protein; lipid fluidity.

Effect of Betaine on the Activity of Three Tumor Markers LDH, ALD, CK in Serum of Mice Bearing S180

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Objective: To study the anti-tumor activity of betaine and its effects on the activity of tumor markers LDH, ALD, CK in serum.
Method: On the basis of mice bearing S180, select tumor inhibition micee. The enzyme activity of LDH, ALD, CK in serum as indexes, which are determined by the kit test.
Results: The tumor inhibition micee of betaine of different dosages are 39.8%、25.7%、17.7% respectively, betaine of high dosage can remarkably decrease the activity of LDH、ALD、CK, $P<0.01$. Betaine of median and low dosages can decrease the activity of LDH、ALD、CK, $P<0.05$.
Conclusion: Betaine can inhibit the growth of tumor cells in mice bearing S180, its effect has correlation with dosage. Meanwhile, it can decease the activity of LDH, ALD, CK in serum, which is probably the potential mechanism of its anti-tumor effect.
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Effects of Solanine on Km and Vmax of NATase in HepG2 Cells

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Objective: To explore the effects of solanine on Km and Vmax of NATase of HepG2.
Method: Employing HPLC, using 2-AF as substrate, taking concentration of 2-AF as concentration of substrate, in intact HepG2 cells and their cytoplasm, taking the speed of 2-AF being acetylated to 2-AFF by NATase as the rate of NATase, using double reciprocal plot, taking 1/S (the reciprocal of concentration of 2-AF) and 1/V (reaction rate of NATase) as coordinates, got regression equation, calculated Km and Vmax.
Results: Study on enzyme kinetics demonstrated, as for intact HepG2 cells, Km and Vmax of control group were 2.37×10-3±8.37×10-5mM, 9.16×10-4 ±7.54×10-5nmol/106cells, Km and Vmax of the solanine group were 2.22×10-3±9.05×10-5mM, 5.14×10-4 ±3.72×10-5nmol/106cells. As for the cytoplasm of HepG2 cells, Km and Vmax of control group were 8.95×10-3 ±2.61×10-4mM and 2.55×10-6 ±1.92×10-8nmol/min.mgprotein, Km and Vmax of the solanine group were 9.48×10-3±3.63×10-4mM and 2.43×10-6 ±1.32×10-8nmol/ nmol/min.mgprotein, statistically, as for intact HepG2 cells and their cytoplasm, there was no difference between the Km of control group and that of solanine group, but there was remarkable difference between Vmax of control group and that of solanine group, $p<0.001$ for intact cell and $p<0.05$ for cytoplasm.
Conclusion: Solanine is a non-competitive inhibitor of NATs.
Key words: solanine; Km; Vmax; NATase
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Analysis of p53 Gain-of-Function mutations in Transgenic Mouse Salivary Tumors

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p53 is an important tumor suppressor that controls cell cycle progression and apoptosis through transcriptional regulation of a variety of downstream target genes. The p53 gene is mutated in ~50% of all human cancers, and most of the p53 mutations are missense point mutations. Although these mutations generally abrogate the sequence-specific DNA binding function of the wild-type p53 protein, some of them appear to have acquired novel functions that loss-of-function mutations do not have, which confer enhanced tumorigenicity, increased genomic instability, and resistance to cancer chemotherapeutic agents. Although many studies have been done to determine the oncogenic effects of these mutations in cell culture systems, few studies have been performed to dissect their activities during tumor development in in vivo settings. We have previously characterized mammary and salivary tumors arising in MMTV-v-Ha-ras (MMTV-ras) mice and MMTV-ras/p53-/- mice (which produce no p53 protein), and have demonstrated that p53 deficiency results in greatly accelerated tumor onset, increased tumor proliferation rates, increased genetic instability, and decreased sensitivity to Adriamycin. To evaluate the effects of a p53 gain-of-function mutation on tumor properties, we have now interbred MMTV-ras transgenic mice to p53 knock-in mice carrying the R172H gain-of-function mutation. Mice of the MMTV-ras/p53R172H/R172H genotype are being characterized with regard to age of tumor onset, tumor growth rates, and apoptotic levels, and these properties are being compared to those of the MMTV-ras and MMTV-ras/p53-/- tumors. Tumor responses to Adriamycin treatment are also being evaluated. To explain possible differences in tumor properties, gene expression profiles of the three groups of tumors are being characterized and compared to each other by microarray analysis. Results from these studies will be presented.

Anti-cMet One-armed 5D5 Antibody Inhibits Orthotopic Pancreatic Tumor Growth and Improves Survival

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Hepatocyte growth factor (HGF) stimulates proliferation, branched morphogenesis, invasive growth, and angiogenesis via the receptor tyrosine kinase c-Met. HGF/c-Met activity is implicated in driving proliferation and metastasis in numerous cancers. In this study we used a monovalent, one-armed antibody against c-Met (OA-5D5) that specifically blocks HGF binding and c-Met activity. *In vitro* OA-5D5 inhibits HGF/c-Met activity in the KP4 human pancreatic tumor cell line. OA-5D5 was tested *in vivo* in the KP4 pancreatic orthotopic model. Twelve days after orthotopic injection into nude mice, tumor volumes were measured by ultrasound and mice were separated into two groups (49.7±7.1 vs. 49.5±8.2 mm^3, n=13 per group). Mice were treated with OA-5D5 (30 mg/kg, twice weekly, i,p.) or vehicle for two weeks. Following treatment, ultrasound measurements were repeated, mice were euthanized and tumors weighed and measured by digital calipers. OA-5D5 treatment significantly reduced tumor

volume measured by ultrasound (817.5±167.1 vs. 1419.9±205.1 mm^3, P=0.0314). OA-5D5 diminished tumor weight by 54% (1.02±0.20 vs. 2.23±0.27 g, P=0.0016) and tumor volume measured by caliper by 52% (P=0.0033). Tumor volume measured by ultrasound correlated with tumor weight (r=0.935, P=0.0001). Survival studies with the same model were done monitoring survival for 3 months (n=24 per group). OA-5D5 also significantly increased survival (50.0% vs. 25.0%; Logrank (Mantel-Cox) analysis P=0.0229). These results demonstrate that OA-5D5 not only inhibited orthotopic KP4 tumor growth but also improved animal survival, suggesting that the OA-5D5 antibody could have therapeutic value in pancreatic cancer and other cancers driven by HGF/c-Met activity.

The Therapeutic Uses of Stem Cells: Article Review

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The discovery of adult tissue specific stem cells, such as haematopoietic stem cells, which have the ability to "transdifferentiate" into other tissues, has generated much excitement among cell biologists and transplant clinicians. Stem cells that give rise to blood cells and move between bone marrow and peripheral blood are the best-characterized adult stem cells in humans. Recent data suggest that adult stem cells generate differentiated cells beyond their own tissue boundaries, a process termed "developmental plasticity. Adult human stem cells that are intrinsic to various tissues have been described and characterized, some of them only recently. These cells are capable of maintaining, generating, and replacing terminally differentiated cells within their own specific tissue as a consequence of Hematopoeitic physiologic cell turnover or tissue damage due to injury. It carries important implications for the treatment of many liver, heart, and neurodegenerative diseases. They could be also, used to cure patients suffering from spontaneous or injuries-related diseases that are due to particular types of cells functioning incorrectly, such as cardiomyopathy, diabetes mellitus, renal failure, osteoporosis, cancers, Parkinson's disease, spinal cord injuries or genetic abnormalities. Currently, these diseases have slightly or non-efficient treatment options and millions of people around the world are desperately waiting to be cured. Umbilical cord blood stem cells are used as a part of the therapy regimen for nearly 50 diseases today. Therapeutic cloning is a technique that would be used to produce cloned embryos, but only to create stem cells that can in turn be used to repair damaged or defective tissue in the parent of the cloned cells. Such stem cells could theoretically be used to grow replacement livers or hearts (or any of a variety of organs) for transplant without fear of rejection. They might be used to create healthy nerve cells for people with Alzheimer's or Parkinson's disease. Skin cells could be derived from cloned stem cells for burn victims. The new concept of "regenerative medicine" is unprecedented since it involves the

regeneration of normal cells, tissues and organs which could allow treating a patient whereby both, the immediate problem would be corrected and the normal physiological processes restored, without any need for subsequent drugs. This review is focus on potential therapeutic applications of adult stem cells.

Tumor Suppressive Protein Grim-19 Associates With Serine Protease Htra2 for Promoting Cell Death

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Tumor suppression is exerted by multiple cellular factors that ultimately impinge on the cell cycle and/or apoptotic machineries. The interferon (IFN) family of cytokines inhibit tumor growth in vivo and in vitro. Combination of IFN with vitamin A related compounds known as retinoids produces stronger therapeutic efficacy than single agents on several human cancers. We and others have shown that this combination induces apoptosis for suppressing tumor growth. Using a genetic technique, we have described the isolation of a novel cell death regulatory protein GRIM-19. To further understand its mechanism of action, we have employed a yeast-two-hybrid screen and searched for its cellular partners. These studies identified HtrA2, a serine protease implicated in cell death control. HtrA proteases were originally implicated in mediating cellular stress-responses in bacteria. Some of the human homologues of HtrA proteins are also upregulated during ischemic damage. Here we show that GRIM-19 physically interacts with HtrA2 and augments cell death in an IFN/RA dependent manner. Catalytically active HtrA2 was required for promoting a synergistic cell death response induced by IFN/RA. In the presence of GRIM-19, the HtrA2 driven destruction of the anti-apoptotic protein XIAP is augmented. Furthermore, these interactions were disrupted by a HHV-8 coded oncoprotein, vIRF1, which conferred resistance to IFN/RA-induced cell death. This report shows a critical role for HtrA2 in a cytokine-induced cell death response for the first time and its inactivation by a viral protein.

The Grims: Cytokine Driven Novel Suppressors of Tumor Growth

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Cytokines play a major role in preventing the tumor development and progression in vivo. In particular, the Interferon family of cytokines regulates tumor growth by establishing an immune surveillance network and by directly inhibiting tumor growth. An interference with the expression or functioning of IFN receptors and/or the downstream signal transducers results in the development of tumors. We have been

investigating molecular mechanisms involved in Interferon and retinoid induced tumor growth suppression. Using a mammalian genetic approach we have identified several novel cellular Genes-associated with Retinoid-Interferon induced Mortality. Some of these GRIMs modulate the activities of cellular apoptotic machineries such as the caspases and others inhibit the activity of oncogenic transcription factors. Thus, the GRIM network forms a new interface between cell death regulators and exogenous growth inhibitors. Earlier, we have characterized a novel gene product GRIM-19 whose experimental inactivation promotes tumor growth. The pro-apoptotic effects of GRIM-19 are inhibited by viral oncoproteins. GRIM-19 inhibits the oncogenic transcription factor STAT3 for promoting apoptosis. STAT3 is constitutively activated in a number of human tumors. We have recently initiated a study for defining the molecular changes associated with human cancers. We have identified that tumor cells accumulate mutations in the GRIM-19 gene, which inactivate its anti-STAT3 activities. We propose that dysregulation of STAT3-inhibitors plays a major role in tumor growth progression. Thus, inactivation of GRIM may act as a key step tumor growth progression and metastasis. We have also developed therapeutic strategies based on GRIM-19 and STAT3 for effective tumor growth suppression.

Methylation Density of RASSF1A and SEMA3B TSG(s) in Renal, Breast, and Ovarian Tumors was found to Correlate with Cancer Progression;

Methylation changes can Contribute to Activation of RHOA oncogene in Tumors

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The methylation of SEMA3B and RASSF1A TSGs and RHOA oncogene was assayed in primary tumors of Muscovites with renal cell (RCC, 70 cases), breast (BC, 60 cases) and epithelial ovarian cancer (EOC, 50 cases). Methylation-specific PCR (MSP), bisulfate sequencing, and methylation-sensitive restriction enzyme analysis (MSRA) were applied. Hypermethylation of RASSF1A promoter region was revealed the most frequently (>60%) in three cancer types. The second distal promoter CpG island was identified in SEMA3B 5’-region, 1.4 kb upstream to the first proximal CpG island, reported earlier. We found frequent hypermethylation of the new CpG island of SEMA3B in range 35-

50% in RCC, BC and EOC, and we confirmed significant correlation of this CpG island methylation with transcription level of this gene, analyzed by RT-PCR. Of importance, high methylation density of RASSF1A gene was found to be associated with high grade and stage of RCC and EOC ($p<0.001$). Methylation density of the new distal SEMA3B CpG island was found to correlate with grade and stage of all three cancers analyzed ($p<0.001$). Association of high methylation density of RASSF1A and SEMA3B promoter CpG islands with tumor progression can be used as prognostic and monitoring tests for patients with RCC, BC, and EOC. Besides, we reported here methylation density changes in promoter region of RHOA putative oncogene in epithelial tumors (in 23 of 45 BC, RCC and EOC cases in total). Decrease of RHOA methylation density in tumor DNA was observed most frequently (15/45 vs. 8/45), and RHOA demethylation was associated with elevated transcription ($p<<0.001$). Therefore, methylation changes can contribute to activation of RHOA oncogene in tumors.

Clinicopathological Features and Genetic Expressing Profile in Hepatocellular Carcinoma in a Morrocan Prospective Study

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Introduction: Hepatocellular carcinoma (HCC) is the one of the most common causes of cancer-induced death in the world. The aim of this study is to describe the clinicopathological profile of HCC and to correlate them of the genetic alterations accumulated during the process of hepatocarcinogenesis.
Patients and methods: This study was conducted between January 2003 and January 2006 on Moroccan patients affected by HCC, the clinical information will reveal. For all patients, samples or surgical pieces were fresh frozen in nitrogen liquid and sent to Pasteur Institute in Paris for genetic analysis: After DNA extraction, sequencing exons and screening polymorphisms have interesting point of mutations (P53, bcatenin, KRAS) and loss of heterozygosity (LOH :including 13 chromosomes and allelic fractional loss (FAL) were high over than 15%).the correlation of clinical features (age, gender, tumors size, number of tumors nodules, histological differentiation, risk factors, vascular thrombosis) were analyzed.
Results: There were 26 patients with HCC, mean of age: 57, 5± 28, 5, sex-ratio: 2, 3 (men/women). In background of patients: low alcohol consumption in 5 / 26 (19, 2%), diabetes non –insulin – dependent in 4 /26 (15, 4 %), muscular injection in 7/26 (26, 9 %), dental cares in 12/26 (46, 15%), surgical anterior treatment in 6/26 (23%) Liver cirrhosis was affecting patients in 22/26 (84, 6%), hepatitis C in 22/26 (84, 6%) and seropositive AFP in 13/26 (50%). The

tumor size was 2,4 ± 5,6 cm , number of tumors nodules 2,3± 5,7 , vascular thrombosis was present in 6/26 (23%) lymphatic regional metastasis in 2/26 (7,7 %) and extrahepatic metastasis in 1/26 (3,8 %). The HCC was highly differentiated in 11/26 (24, 3%).LOH were in 19/26 (73, 1%); P53 was muted in 5/25 (19, 2 %); bcatenin in 3/26 (11, 5%) and no KRAS mutation was found. High FAL was correlated to a bigger size HCC (ñ 3cm) (p á 0,005) with more than 3 tumors nodules.

The Nuclear EGFR in Bladder Cancer: Trafficking and Regulatory Mechanisms of its Transcription Function

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A number of reports have demonstrated that the EGF receptor (EGFR) can accumulate in the nucleus in certain pathological situations. Nuclear EGFR has been shown to perform signaling functions distinct from the role of the protein as a growth factor receptor, including cooperation with other proteins in the formation of transcriptional complexes. Because EGFR is a cognate receptor for HB-EGF, which accumulates in the nucleus in aggressive transitional cell carcinoma (TCC; Cancer Res. 63:484, 2003; Cancer Res. 65:8242, 2005), the possibility that nuclear EGFR plays a role in bladder cancer was assessed. Automated quantitative analysis (AQUA) of a bladder cancer tissue microarray (TMA), consisting of 18 tumors and 3 normal tissues, demonstrated that levels of nuclear EGFR were increased in bladder cancer tissues relative to benign tissues (p=0.003), however, no such correlation was observed with cytosolic EGFR (p=0.12). HB-EGF stimulated EGFR trafficking to the nucleus and EGFR binding to the cyclin D1 promoter in a PI3-kinase- and Erk/MAPK-dependent manner. Nuclear localization of EGFR was biphasic, with rapid accumulation in the nucleus followed by apparent degradation of the protein. Analysis of anti-EGFR immunoprecipitates by mass spectrometry identified the phosphoinositide 5-kinase, PIKfyve, and cullin-2, a ubiquitin ligase, as potential components of the EGFR trafficking complex. RNAi-mediated PIKfyve protein depletion or expression of the dominant-negative kinase-deficient PIKfyveK1831E mutant reduced EGFR nuclear localization, whereas ectopic expression of PIKfyveWT elevated levels of nuclear EGFR. A proteasome

inhibitor (MG132), but not a lysosomal inhibitor (ammonium chloride), suppressed degradation of nuclear EGFR, suggesting that accumulation of nuclear EGFR is regulated by a proteasome-dependent mechanism. Association of cytosolic and nuclear EGFR with cullin-2 correlated with the kinetics of EGFR ubiquitination, suggesting a functional role for cullin-2 in the control of intracellular EGFR levels in both cytoplasm and nucleus. These data demonstrate that (1) nuclear EGFR appears to play a physiologically relevant role in TCC; (2) PIKfyve plays a direct role in EGFR trafficking to the nucleus; (3) cullin-2 may be a novel mediator of proteasomal degradation of EGFR during trafficking from the cytosol to the nucleus. These observations provide evidence that EGFR, which is overexpressed in bladder cancer, plays a pleoitropic role in signaling and transcriptional regulatory mechanisms of tumor cell proliferation. This study has also identified two new potential molecular targets for directed bladder cancer therapy.

The Relationship between the Expression of MHC Class II Antigens and the Clinical Prognosis in Papillary Thyroid Carcinoma

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Papillary thyroid carcinoma is among the most curable cancers. However, some patients are at high risk for relapse and distant metastasis or even death. MHC antigens are essential molecules for pathogenesis of the carcinoma and human physiologic immune responses against tumor. In this study, we investigated the correlation between the expression of MHC antigens and clinical prognosis in papillary thyroid carcinoma. The patients were 215 who had undergone thyroidectomy between 1987 and 2003 and we analyzed the relationship between the clinical prognosis and various prognostic factors with MHC antigen expressions by retrospective study. The expression of MHC class II antigens were higher in papillary thyroid carcinoma than other thyroid diseases. But, there was no statistical significance in most clinicopathological factors according to the MHC class II antigen positivity in papillary thyroid carcinoma. No statistical significance was found between the MHC class I antigen expression and clinicopathological relapse. Interestingly, HLA-DR expression of MHC class II antigen was found in 8 (30.8%) of the 26 patients in relapse group and 13 (76.5%) of 17 patients in non-relapse group. HLA-DP/DQ immunoreactivity was positive in 10 (38.5%) cases in relapse group and 14 (82.4%) cases in non-relapse group. Multiple regression analysis showed that HLA-DR (OR = 0.137, 95% CI; 0.034~0.552, $P < 0.01$) and HLA-DP/DQ (OR = 0.134, 95% CI ; 0.031~0.586, $P < 0.01$) expression were the significant determinants of the

relapse of the papillary thyroid carcinoma. As the relapsed papillary thyroid carcinomas have a tendency to down regulate the expression of MHC class II antigens, the MHC class II antigen may be the molecular marker of the prognosis of papillary thyroid carcinoma. However, larger, randomized prospective study may be needed for better understandings of the relationship between the expression of MHC antigens and clinical prognosis in papillary thyroid carcinoma.

Oncogenic Action of Kras in Mouse Models

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We use a binary system of Cre-producing and Cre-responding mice for cancer modeling at chosen anatomical sites. Depending on the tissue-specificity of the promoter driving Cre expression, tumors develop in bitransgenic progeny by crossing Cre-producers with partners carrying in the highly transcribed Eef1a1 recipient locus a dormant, “knocked-in” oncogene, such as KrasG12D (Kras*), placed downstream from a “floxed” stop sequence. In breeding programs using various Cre-producers to excise the floxed block, we observed that activation of Kras* overexpression caused development of tumors in mammary and salivary glands, pancreas, prostate, skin and the hematopoietic system, which are now being analyzed to identify common and unique molecular consequences of Kras* action. For example, unsupervised analysis of expression profiling data showed a striking pattern of similarity between Kras-induced mammary and pancreatic carcinomas. Kras* activation in lactating mammary glands by a WAP-driven Cre transgene resulted unexpectedly in extremely rapid development of invasive breast tumors detected as early as 2 days after the first delivery (T50=18 days). Apparently, overexpression of Kras* was able to elicit a combinatorial deregulation of downstream pathways to a degree sufficient for development of malignancy in a single step. Thus, this model can be used for dissecting genetically collaborating tumorigenic pathways and potentially identifying drug-targets. Notably, members of key signaling pathways, such as Igf1r, Egfr and Notch1, were upregulated in the tumors. Breast-specific ablation of Igf1r or Myc prevented tumor development. We have previously shown that Myc is a direct transcriptional target of Notch1 and an essential effector in mammary oncogenesis induced by overexpression of the constitutively active intracellular Notch1 domain. We are investigating whether the Myc involvement in Kras* tumors occurs through Notch1 signaling potentially functioning downstream of Kras.

The use of Fluorescent Probes Combined With Quantum Dots for the Study of Tumor Progression and Therapeutic Effects

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A cell fluorescence imaging method on a CCD camera operated on two modes (real time, frame accumulation mode) has been used in combination with a frame grabber to evaluate the cell's organelles. The fluorescence probes are specific organelle probes and nanomethod probes called quantum dots. Tumor progression and drug effects are both expected to produce alterations in the mitochondria, endoplasmic reticulum, golgi apparatus and lyosomes of malignant cells. The use of nanomethod probes as quantum dots enhances the sensitivity and spectral versatility of the method. While organelle selective probes have been used extensively, the application of the quantum dots to tumor cells is in a very preliminary stage. Usually both in yeast and tumor cells, the quantum dots remain localized on the cell membrane but trials are planned to allow penetration and localization of these probes and also immunolabeling.

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Integrin Profiling: Diagnostic and tTherapeutic Opportunities

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In cancer, integrins are involved in loss of anchorage-dependent growth, tumor angiogenesis and tumor cell migration during tissue invasion and metastasis. Integrin functions are important in nearly every tissue and knowledge is far from complete. Cells usually express more than one integrin, in complex patterns that can change with time and environment and overlap between cell types. Because some integrins are upregulated in both invasive cancer cells and angiogenic endothelial cells, their targeting might confer a significant therapeutic advantage. Immunohistochemical profiling of the integrins provides helpful information for the selection of candidates for these clinical trials.

Cyclooxygenase-2 Plays Crucial Role in Osteopontin-induced PKC alpha/IKK alpha/beta dependent Prostate Tumor Growth and Angiogenesis

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Cell migration and degradation of extracellular matrix are important steps in tumor progression. Here we report that osteopontin (OPN) a SIBLING (small integrin-binding ligand N-linked glycoprotein) family of ECM-associated

protein regulates prostate tumor growth by inducing the expression of cyclooxygenase-2 (COX-2). OPN stimulates the activation of PKC alpha/NIK/NFkB dependent signaling cascades that induces COX-2 expression, which in turn regulates the PGE2 production, MMP-2 activation, tumor progression and angiogenesis. The data revealed that suppression of OPN-induced COX-2 expression by nonsteroidal, anti-inflammatory drug, celecoxib or blocking the EP2 receptor by its blocking antibody resulted significant inhibition of cell motility, tumor growth and angiogenesis. OPN-induced mice PC-3 xenograft exhibits higher tumor load, increased tumor cell infiltration, nuclear polymorphism and neovascularization, however using of celecoxib or anti-EP2 blocking antibody drastically suppressed OPN-induced tumor growth suggesting that suppression of COX-2 or its metabolites could significantly inhibit OPN-induced tumor growth. Human clinical prostate cancer specimen analysis also supports the in vitro and animal model studies. These data suggest that blockage of OPN and/or COX-2 might be a potential therapeutic target for the treatment of prostate cancer.

Determinants of Chemo- and TRAIL Sensitivity in Esophageal Cancer Cell Lines

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More than 85% of the patients die within 2 years after the diagnosis of advanced esophageal cancers. One of the reasons is they develop resistance to chemo- and radiotherapy, and thus understanding and overcoming the anti-apoptotic mechanisms of therapeutic resistance in esophageal cancer is an important problem. In this study we used panel of 14 esophageal squamous cancer cell lines (TE1, TE2, TE3, TE5, TE7, TE8, TE9, TE10, TE11, TE12, TE15, TT, HCE4) as well as the adenocarcinoma line HCE7. Sensitivity of those lines to TRAIL, 5FU, carboplatin, and taxol were tested using a colony formation assay. To delineate factors relevant to sensitivity or resistance to the above treatments, the expression levels of TRAIL receptors DR4, KILLER/DR5, DcR1, DcR2, as well as other modulators of apoptosis, proliferation or cell survival including Bcl2, BclXL, CIAP1, XIAP, cyclin D1 and EGF receptor (EGFR) were examined by immunoblotting. Cell lines TE1, TE2, TE9 and TT were found to be sensitive to TRAIL treatment. These 4 cell lines expressed high levels of DR4 protein while seven of ten TRAIL-resistant cell lines showed lower levels of DR4. Three of four TRAIL-sensitive cell lines showed lower levels of DcR1, whereas seven of ten TRAIL-resistant lines showed higher levels of DcR1. TE12 and HCE7 cells were highly resistant to TRAIL and chemotherapy. These two cell lines expressed Bcl-XL whereas only one of twelve chemosensitive cell lines expressed Bcl-XL. The EGFR was highly expressed in TE3, TE11 and TE12 cells which were resistant to 5FU and carboplatin but not to taxol. Our studies are providing insights into the molecular mechanism(s) of TRAIL and

chemotherapeutic sensitivity and resistance in esophageal cancer.

Vaccination with EphA2-derived T Cell-epitopes Promotes Immunity against Both EphA2-expressing and EphA2-negative Tumors

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Background: A novel tyrosine kinase receptor EphA2 is expressed at high levels in advanced and met static cancers. We examined whether vaccinations with synthetic mouse EphA2 (mEphA2)-derived peptides that serve as T cell epitopes could induce protective and therapeutic anti-tumor immunity.
Methods: C57BL/6 mice received subcutaneous (s.c.) vaccinations with bone marrow-derived dendritic cells (DCs) pulsed with synthetic peptides recognized by CD8+ (mEphA2671-679, mEphA2682-689) and CD4+ (mEphA230-44) T cells. Splenocytes (SPCs) were harvested from primed mice to assess the induction of cytotoxic T lymphocyte (CTL) responses against syngeneic glioma, sarcoma and melanoma cell lines. The ability of these vaccines to prevent or treat tumor (s.c. injected MCA205 sarcoma or B16 melanoma; i.v. injected B16-BL6) establishment/progression was then assessed.
Results: Immunization of C57BL/6 mice with mEphA2-derived peptides induced specific CTL responses in SPCs. Vaccination with mEPhA2 peptides, but not control ovalbumin (OVA) peptides, prevented the establishment or prevented the growth of EphA2+ or EphA2-negative syngeneic tumors in both s.c. and lung metastasis models.
Conclusions: These data indicate that mEphA2 can serve as an attractive target against which to direct anti-tumor immunity. The ability of mEphA2 vaccines to impact EphA2-negative tumors such as the B16 melanoma may suggest that such beneficial immunity may be directed against alternative EphA2+ target cells, such as the tumor-associated vascular endothelial cells.

Investigation of the Relationship between Wnt5a and Claudin-1

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Our previous gene expression studies have identified the proteins Wnt5a and Claudin-1 as upregulated in melanoma. Staining of a human tissue microarray revealed that Claudin-1, which is involved in regulation of the paracellular transport of molecules, was

overexpressed in the cytoplasm of malignant cells, suggesting a role other than transport. Indeed, melanoma cells in culture demonstrate no tight junction function and overexpression of CLDN1 in melanoma cell-lines led to increased motility of the cells, while knockdown of CLDN1 by SiRNA resulted in reduced motility. Using gelatin zymogrpahy and wound-healing assays we showed that the Claudin-1 related motility occurs via the matrix metalloproteinase 2 (MMP-2) pathway. It has been shown that protein kinase C (PKC) can affect expression of Claudin-1 in rat choroids plexus cells, and we observed a correlation between levels of activated PKC and Claudin-1 expression in our melanoma cells. Our other studies have previously shown that increased Wnt5a increases motility of melanoma cells via the activation of PKC. We investigated the association between Wnt5a expression and Claudin-1 expression by examining the endogenous levels of both in melanoma cell lines by real-time PCR and Western blot analysis. Cell-lines that had high Wnt5a levels had high Claudin-1 levels and vice versa. To determine if Wnt5a could directly affect Claudin-1 expression, cells that had low Wnt5a and Claudin-1 expression, were treated with recombinant Wnt5a, and cells with high Claudin-1 and high Wnt5a were treated with siRNA against Wnt5a. Wnt5a treatment was able to increase the levels of Claudin-1 in melanoma cells, and knockdown of Wnt5a by RNAi also resulted in decreased claudin-1 expression. Furthermore, our data indicate that claudin-1 is more aggressive when located in the cytoplasm of the cell, versus the nucleus or membrane, supporting our tissue microarray observations. Confocal microscopy determined that Claudin-1 expression increased in the cytoplasm after treatment with recombinant Wnt5a. Finally, increases in Wnt5a have been linked to increased invasion in melanoma cells, and increases in claudin-1 correspond to increases in the invasion-promoting protease, MMP2. Treatment of non-invasive, low-claudin-1 expressing melanoma cells with recombinant Wnt5a results in the dramatic increase of MMP-2. Taken together these data indicate that the non-canonical Wnt pathway can aberrantly increase the expression of the tight-junction protein claudin-1, causing its mislocalization, and leading to increases in MMP-2, and invasion.

Mechanisms for 15dPGJ2 Sensitization of TRAIL Induced Cytotoxicity: Role of CHOP Mediated DR5 Upregulation

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Even though there have been many advances in the therapy of cancer following the introduction of cytotoxic chemotherapeutic drugs, the development of drug-resistance remains a major obstacle in the treatment of tumors. TRAIL has been shown to be selectively cytotoxic in inducing tumor cell death, and has minimal or no toxicity against normal tissues. Thus TRAIL-mediated tumor cell death, either alone or in combination with other anticancer therapy, is considered as new strategy with great potential for

anticancer effect. In this study we investigated the combinatorial) inγeffects and molecular mechanisms of 15dPGJ2 (an activator of PPAR sensitization of TRAIL-induced apoptosis of human colon cancer HCT116 cells. We induced cell apoptosis. Results αfound 15dPGJ2 can potentiate TRAIL- and/or TNF revealed that the sensitization of TRAIL cytotoxicity by 15dPGJ2 is resulting from the transcriptional upregulation of death receptor 5 (DR5), but independent. We demonstrated CHOP as a mediator of DR5 induction by15PGJ2. CHOPγof PPAR small interfering RNA attenuated the DR5 up-regulation due to 15PGJ2 and reversed its sensitization effects with TRAIL. Moreover, DR5 expression through CHOP up-regulation is mediated by ROS, calcium, PKC and PKR. Furthermore, we also found that 15dPGJ2 is able to induce GRP78 and XBP1 expression, indicating the contribution of ER stress. In summary, the sensitization effect of 15dPGJ2 independent pathway, but depends on CHOP-mediated DR5γis through PPAR upregulation. The increase of ROS, calcium and PKC activity are contributed to CHOP gene transcription.

Dock180/ ELMO-1 Complex Competes with C3G to Activate Cell Motility through Rac1 and its Role in Human ovarian Cancer Cells

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Dock180 was well known for the main target of signal adaptor protein Crk and acting as a guarnine nucleotide exchange factor (GEF) for small GTPase Rac1. Forced expression of Dock180 was found to be associated with Elmo1 and these two molecules were recognized to cooperate together to activate Rac1. It has been largely reported to be associated with cell migration, and its function as a promoter for cell invasion was suggested in some malignancies. In the present study, Dock180 was confirmed to cooperate with Elmo1 in vivo in ovarian cancer cells MCAS and RMUG-L, and CrkI other than CrkII presents preferential combination with it. In Dock180 knockdown cells established by means of siRNA, we found that Elmo1 expression and Rac1 activity were decreased simultaneously, while in contrast, the expression of another Crk-combining molecule C3G was observed to increase obviously, and it was the same with Rap1 activity consequently. Accordingly, all Dock180 knockdown cells present with evident change in cell morphology and reduction in cell migration and invasion. These results suggest that Dock180/Elmo1 complex acts against C3G competitively to combine with Crk, and is implicated in actin cytoskeleton reorganization, and thus in the malignant transformation of ovarian cancer.

Involvement of Adaptor Protein Crk in Malignant Feature of Human Ovarian Cancer Cell Line MCAS

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Signaling adaptor protein Crk regulates cell motility and growth through its targets Dock180 and C3G, those are the guanine nucleotide exchange factors (GEF) for small GTPases Rac and Rap, respectively. Recently, overexpression of Crk has been reported in various human cancers. To define the role for Crk in human cancer cells, Crk expression was targeted in the human ovarian cancer cell line MCAS through RNA interference, resulting in the establishment of three Crk knock down cell lines. These cell lines exhibited disorganized actin fibers, reduced number of focal adhesions, and abolishment of lamellipodia formation. Decreased Rac activity was demonstrated by pull down assay and FRET based time-lapse microscopy, in association with suppression of both motility and invasion by phagokinetic track assay and traswell assay in these cells. Furthermore, Crk knock down cells exhibited slow growth rates in culture and suppressed anchorage-dependent growth in soft agar. Tumor forming potential in nude mice was attenuated, and intraperitoneal dissemination was not observed when Crk knock down cells were injected into the peritoneal cavity. These results suggest that the Crk is a key component of focal adhesion and involved in cell growth, invasion, and dissemination of human ovarian cancer cell line MCAS.

Sympathicus as Cancerkiller

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In the acceptance that the immunedefence is without importance the present idea of cancer is based entirely on intracellular genetic conditions. But this is incompatible with the mathematical laws of probability, which state that species with many cells should have a greater incidence of cancer compared with species with fewer cells – indeed; the opposite is mostly the fact. For instance do humans have about one million times the cell of mice, but with a cancer incidence far below that of mice. The evidences of immediate cell destruction beyond the scope of apoptosis - for instance described in ‘Nature’ - brings about the assumption that extracellular factors are of major importance. The sympathetic nervous system is able, preceded by afferent signals, to act locally, and

noradrenalin has been proven to kill the cancer cells. The metabolism of the cell is accelerated, thereby exhausting and terminating the cell in a couple of days. Further, it is very possible, that the halting of the blood supply seen in mature tumours is due to the physiological effect of noradrenalin on the vessel walls. The fundamental idea comes from the fact that the relative brain size by a cohort of 10 mammalian species, ranging from cattle over man till mice, high significantly has been proven to correlate with the prevalence of spontaneous, generalized malignant tumours by this cohort. (E Lundsgaard. Generalised cancer, a function of the relative Brainsize). This may indicate that the strain on the autonomous nervous system, dispersed throughout the brain, is increasing with the increasing ratio of neurones belonging to 'receiving, processing and conscious areas' - it means, all thing others than sympathetic neurons. The extension or density of sympathicus in the brain is though to be adapted to the dimensions of the body it supplies. Interference in neocortex and other parts of the brain, partly stress-induced but probably conditioned as well, might cause dysfunction of sympathicus, which aggravates with a proportional increase of 'receiving, processing and conscious areas'.

The Significance of TK1 Immunochemistry in Normal Kidney and Renal Cell Carcinoma

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Purposes: Thymidine kinase 1 (TK1) is a key enzyme involved in the synthesis of precursors for DNA, and thus proliferation dependent. Little is known about the significance of TK 1 in renal cell carcinoma. We examined the expression of TK1 and Ki-67 and the serum concentration of TK1 (STK1) to determine the significance and its association with other clinic pathologic parameters such as stage and grade.
Materials and methods: Serum samples from renal cell carcinoma patients were analyzed by enhanced chemiluminescent (ECL) and immunohistochemistry (IHC) respectively; fresh tissues were analyzed by western-blotting.
Results: Significantly mean positive ratio of TK1 in normal kidney was approximately 2-fold higher than in renal cellcarcinoma（P=0); western blotting also confirm that the TK1 expression in normal kidney was approximately higher that in renal cell carcinoma. KI-67 staining in normal kidney is negative, but significantly mean positive ratio of KI-67 in renal cell carcinoma is 0.2599±0.1180（P=0). No statistically significantly difference of the concentration of TK1 in sera exists between the healthy and renal cell carcinoma（P=0.87). The ratios of TK1 and Ki-67 in tissues and the concentration of TK1 in sera in grade 3 were statistically significantly higher than that in grade 1（P=0.009, P=0.007 and P=0.044, respectively).
Conclusion: To our knowledge this report represents the first study to demonstrate that TK1 presents in normal kidney is higher than in renal cell carcinoma. The expression and concentration of TK1 correlate with expression of KI-67 and the clinical grade in renal cell carcinoma. The

expression and concentration of TK1 correlate with expression of KI-67.

Down-Regulation of Cluster Determinants in CRL-2610 Cells upon the Induction of Apoptosis

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This investigation has obtained a profile of the surface cluster determinants (CDs) that are present on CRL-2610 glioblastoma cells. In addition, it was determined how the cell surface density of the detected CDs changed following the induction of apoptosis. Apoptosis was induced by both MK886 and staurosporine. MK886 is a known inhibitor of five-lipoxygenase-activating-protein (FLAP) and peroxisome proliferator-activated receptor antigen alpha (PPAR-α). Staurosporine is a known inhibitor of a number of protein kinases including Protein Kinase C (PKC). The detection and down-regulation of surface CDs were observed by flow cytometry using CD-specific mouse monoclonal antibodies followed by goat anti-mouse-IgG conjugated to the fluorescent dye phycoerythrin. The study focused on CDs involved in the stimulation of immune surveillance, integrins, and growth factors, along with Class-1 histocompatibilty markers. It was determined that there was a down-regulation of the growth factor receptors EGFR and IGFR1 (CD221) on the surface of apoptotic cells. The class-1 histocompatibilty cell surface marker HLA-ABC was also severely down-regulated in the apoptotic cells. This is of interest, for abnormally low levels of class-1 histocompatibility surface determinants can be stimulatory to NK cells. The alpha and beta integrins that were detected include ITGA1 (CD49a), ITGA2 (CD49b), ITGA3 (CD49c), ITGA4 (CD49d), ITGA5 (CD49e), ITGA6 (CD49f), ITGB4 (CD104), ITGB5, and ITGA2B (CD41), all of which dropped dramatically as apoptosis progressed. Overall, the data indicate that cluster determinants are down-regulated in mid to late apoptosis in a manner that can reduce growth and survival receptor mediated signals. Funded by grant EPS 2006-15 and by grant P20 RRi6477 from the National Center for Research Resources awarded to the West Virginia IDeA Network for Biomedical Research Excellence.

Fgf2 Binding, Signaling and Angiogenesis are Modulated by Heparanase in Metastatic Melanoma Cells

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Heparanase (HPSE) and fibroblast growth factor-2 (FGF2) are critical

regulators of melanoma angiogenesis and metastasis. Elevated HPSE expression contributes to melanoma progression; however, further augmentation of HPSE presence can inhibit tumorigenicity. HPSE enzymatically cleaves heparan sulfate glycosaminoglycan chains (HS) from proteoglycans. HS acts as both low-affinity FGF2 receptors and co-receptors in the formation of high-affinity FGF2 receptors. We have investigated HPSE's ability to modulate FGF2 activity through HS remodeling. Extensive HPSE degradation of human metastatic melanoma cells (70W) inhibited FGF2 binding. Unexpectedly, treatment of 70W cells with low HPSE concentrations enhanced FGF2 binding. In addition, HPSE-unexposed cells did not phosphorylate extracellular signal-related kinase (ERK) or focal adhesion kinase (FAK) in response to FGF2. Conversely, in cells treated with HPSE, FGF2 stimulated ERK and FAK phosphorylation. Secondly, presence of soluble HPSE-degraded HS enhanced FGF2 binding and ERK phosphorylation at low HS concentrations. Higher concentrations of soluble HS inhibited FGF2 binding but FGF2 signaling through ERK remained enhanced. Soluble HS were unable to support FGF2-stimulated FAK phosphorylation irrespective of HPSE treatment. Finally, cell exposure to HPSE or to HPSE-degraded HS modulated FGF2-induced angiogenesis in melanoma. In conclusion, these effects suggest relevant mechanisms for HPSE modulation melanoma growth factor responsiveness and tumorigenicity.

Overcoming Drug Resistance of Hypoxic Cancer Cells Using Hypoxia-Specific Small Molecule Sensitizers of TRAIL-Induced Apoptosis

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A hallmark of solid tumors is the presence of abnormal vasculature which causes heterogeneity of tumor nutrient and oxygen supply. The result is regions of tumor necrosis and hypoxia. These factors pose significant barriers to treating solid tumors and are responsible for difficulties of drug delivery and more importantly for issues of therapeutic resistance. Here we demonstrate the use of hypoxia-specific small molecule sensitizers to TRAIL-induced apoptosis as a strategy to overcome the therapeutic resistance of hypoxic HCT116 colon carcinoma cells. A library of small molecules was screened against p53-null HCT116 cells in a high-throughput manner using cell-based bioluminescence imaging. Unlike hypoxic HCT116 p53 wild-type cells, p53-null cells remain resistant to combinations of TRAIL with chemotherapeutics, including the

hypoxia-activated topoisomerase II inhibitor (Tirapazamine), kindly provided by J. Martin Brown of Stanford University. Therefore, we screened for small molecules capable of specifically sensitizing hypoxic HCT116 p53-null cells. Here we show the identification of small molecules capable of sensitizing these cells to TRAIL-induced apoptosis. Follow-up studies of lead-candidates from the primary screen are currently underway to access structure-activity relationships between molecules as well as identifying the molecular targets responsible for sensitizing affects. This study demonstrates the importance of accounting for physiologic factors within the tumor microenvironment, such as hypoxia, when discovering new agents for drug development.

Prediction of Oligodendroglioma Chemotherapy Response by Microarray Profiling Of DNA, RNA, microRNA, and Methylation Status of Tumor

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Inter-individual differences of treatment response to cancer therapy have become a challenging problem. The new field of pharmacogenomics, using DNA microarray analysis, focuses on the genetic determinants of drug response at many levels: DNA, RNA, Protein and epigenetic factors. DNA modifications of mutations, amplifications, and deletions, RNA expression levels including micro RNA expression, changes in protein content, methylation status difference and combinations thereof can be explored globally to elucidate their component in diagnosis and treatment outcome. For example oligodendrogliomas comprise more than 20% of all gliomas (the most common primary central nervous system tumors in adults). Compared with most other gliomas, oligodendroglioms have a better prognosis. Although oligodendrogliomas are more sensitive to chemotherapy, the response rate to chemotherapy is 40–70%. To better understand this inter-individual variability response we used resected specimens of oligodendrogliomas before treatment with the oral alkylating agent temozolomide. Patients were followed with serial brain MRI scans every two months to record time to progression. Time to progression is defined as the time required for interval increase in tumor size by greater than 25%. We conducted global gene expression profiling, Methylation profiling, miRNA profiling and global comparative genomic analysis to predict personalized chemotherapy response.

Down Regulation of Wip-1 Phosphatase in MCF-7 Breast Cancer Cells Enhances Doxorubicin-induced Apoptosis Through p53-mediated Transcriptional Activation of Bax

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The PPM1D gene localized within the 17q22-23 amplicon encodes a type 2C-like phosphatase (Wip-1). It was first identified as a gene induced in cells exposed to UV- and IR-radiation in a p53-dependent manner. The gene is amplified in 12 to 20% of primary human breast, ovarian and neuroblastoma tumors most of which also harbor a wild type p53 gene. The human breast cancer cell line MCF-7 carries an amplified PPM1D gene and harbors a wild type p53 gene. Nuclear Wip-1 phosphatase protein is express at elevated levels in these cells. We established stable isogenic cell lines (MCF-Sp53 clones) which carry a pSuppressor/p53 plasmid and exhibit markedly decreased levels of wild type p53 protein following exposure to the anticancer drug doxorubicin. We show that although the PPM1D gene is amplified in MCF-7 cells it is still expressed in a p53-dependent manner. In addition, stable isogenic cell lines derived from parental MCF-7 cells (designated MCF-clones) were established in which Wip-1 expression is significantly down regulated by constitutive expression of a plasmid-based PPM1D antisense RNA. Down regulation of Wip-1 expression sensitizes MCF-clones to doxorubicin-induced apoptosis. The enhanced apoptotic response is correlated with increased phosphorylation of N-terminal p53-Ser15 and -Ser46 and increased expression of the endogenous p53 responsive pro-apoptotic Bax gene at both the mRNA and protein level. The enhanced apoptotic response in MCF-clones was blocked by Bax-siRNA knock down suggesting that the increased response was a result of increased Bax protein expression. Moreover, reporter gene assays using the Waf-1 and Bax promoters to drive a luciferase gene revealed that luciferase activity driven by the Bax promoter was enhanced in MCF-clones while luciferase activity driven by the Waf-1 promoter was decreased relative to parental MCF-7 cells. The present study reveals a novel molecular mechanism involving Wip-1 phosphatase, phosphorylation of p53 N-terminus Ser15 and Ser46 and an enhanced apoptotic response mediated by transcriptional activation of the pro-apoptotic p53 target gene Bax. This research was supported by NIH grant 1 R01 CA 79756-04 to WEM

Quantum Chemical DFT Calculations of IR Vibrational Spectra of Steroid Hormones

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Human sexual hormones such as estradiol, estriol, testosteron, methyltestosteron, ethyniltestosteron are calculated by the most accurate method of modern quantum chemistry: density functional theory (DFT) with B3LYP functional and 6-31G** basis set. The structure of molecules and their infrared (IR) spectra are calculated. For the first time we provide complete interpretation of all vibrational modes including fingerprint regions. The role of low-frequency vibrations in hormone-receptor interaction is stressed. The treatment of breast cancer and the cause of the disease are intricately linked to the activity of the estrogen receptor and the normal blood serum level of 17beta-estradiol. The model of binding of this hormone with estrogen receptor is discussed on the basis of theoretical data on low-frequency vibrations, which provide essentila change of the effective volume of hormone molecule.

A Proteomic Analysis Reveals that the STAT3-Inhibitor GRIM-19 is a Novel Tumor Suppressor of Human Renal Cell Carcinoma

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Interferons (IFN) and retinoids (vitamin related compounds such as retinoic acid) are potent tumor growth inhibitors. We showed that IFN/RA combination synergistically suppresses tumor growth by inducing apoptosis. Using a genetic technique we have identified a novel gene product GRIM-19 (Gene associated with Retinoid- Interferon induced Mortality-19) whose inactivation promotes tumor growth. GRIM-19 inhibits the oncogenic transcription factor STAT3 for promoting apoptosis. We have recently initiated a study for defining the molecular changes associated with human renal cell carcinoma by comparing the proteomes of normal and tumor kidneys from patients using mass spectrometry. These studies identified a loss of GRIM-19 expression in the tumors. Using a number of human tumor samples we show a loss of GRIM-19 correlates with tumor growth promotion. Employing a surrogate animal model we show that downregulation of GRIM-19 promotes tumor growth in vivo. These effects are in part mediated via an inhibition of anti-STAT3 activity of GRIM-19. This is a first study that shows a mechanism by

which STAT3 deregulation occurs in human tumors.

Tumor Diagnosing and Targeted Non-Toxic Treating Based on Malignant Parasitism for Essential Bioantioxidants

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Uncontrolled autonomic growing, invasiveness and metastasing are biologic features for malignancies. All specifics of tumor progression are provided with biochemic parasitism which becomes health significant when essential bioantioxidants (BAO) as vitamins E and A are involved. Malignant BAO parasitism. Experimental tumors were discovered as the trap for vitamins E (vE) and A (vA) with following general mechanisms: 1) blastoms secrete factors of 2 types to bloodstream and tumor - surrounding tissues to mobilize vE and vA from host' organs and tissues; 2) vE esters from organs' pulls are activated to BAO and are mobilized to bloodstream as free vE with peak increase coinciding with peak velocity for tumor growth; 3) tumors trap BAO to co-oxidize them at substrate quantities to prevent there any appearance of lipoperoxic catabolites (LP), natural inhibitors for mitosis (gist of the BAO parasitism); 4) paraneoplastic toxic hyperlipoperoxidation for host's organs and biofluids is developed due to their BAO deficits. Glucose enhances oxidative vE utilization significantly by cancer cells. Tumor-bearers are paralyzed during pure O2 inhalation due to quick poisoning with by order increased LP catabolites, in brain especially. Autonomic tumor growth is supported with BAO parasitism. Due to malignant BAO parasitism tumor-adjoined tissues have subnormal BAO minimum levels and as sequels overnormal maximum levels for LP, which are known membranolytics. These specifics are proposed to take part in tumor invasiveness and metastasing. The same mechanisms were registered for clinic manifestations. Tumor diagnosing & risk evaluation. Simultaneous early BAO- and LP-hyperemias with their specific molar ratia during malignant growth have permitted to develop the PC - Program for differential diagnose of patients with lung tumors by some types and from non-specific lung chronic diseases (NLCD) with close symptomatics, as from norms. Individual relative risks of lung tumors for NLCD patients were evaluated by the same PC - Programmed principle. Tumor-targeted chemotherapy. Being erected on the malignant BAO parasitism objectively, many recent publications have proved apoptosing of cancer cells and tumor-targeted necrotizing with "redox - silent" tocopherols (RST) as vE with esterified hydroxyl, being blocked as BAO into tumors only. RST accumulation into tumors as vE but without ability to be oxidized as BAO (as a broken key in a lock) provokes oxidative stress in mitochondria of actively respiring tumors. But they are splitted to vE and f.i. succinate in host's organs, lacking of the BAO for tumor-bearers. If so, RSTs are the sole powerful antineoplastic medicaments, not only non-toxic but healthy for tumor-bearers as such. Either

tumors are not expected to be RST resistant (or are able to develop resistance) as discovered vE parasitism is their life-necessary activity. RST high benefits open luring perspectives for cheap non-toxic treatment of various cancers with their metastases and hosts simultaneously as systemic “malignant disease” clinically or out quite possibly.

Paneth-Like-Cells Prostatic Adenocarcinoma with Neuroendocrine Differentiation: An Unusual Tumor of an Unknown Progression Potentiality

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We report on a case of prostate adenocarcinoma with foci of Paneth-like-cells prostatic revealing neuroendocrine differentiation (PLC/NEC), in a 73-year-old congolese man evacuated from a rural hospital in august 2002 for a sudden urinary retention which appeared four weeks ago. He was admitted in the Department of Urology and Andrology of the University Hospital Centre of Brazzaville (Congo) where clinical evaluation confirmed an irregular prostate hypertrophy both by digital and sus-pubian echography investigations. PSA test was up to 180 ng/ml ; systematic biological screening (ionogram, hemogram, and renal functions investigations) revealed no particularities; and skeleton radiography was normal. A urinary catheterizing has been released; furthermore, a decompressive adenomectomy which revealed the extension of the tumor to the deep soft tissue has been undertaken. It has been followed by clinical improvement with the relapse of urinary symptoms free of urinary catheter within two years. Meanwhile, the patient died on May 2004 in his village, in imprecise clinical circumstances. The revisiting of the last six-year-histologically-diagnosed prostate cancers (78 cases) results in the current case. The present diagnosis modified two former diagnoses, successively, of not-otherwise-specified adenocarcinoma, and of foamy cells prostatic adenocarcinoma (Gleason score 4+5), which unexpectedly were positively stained with neuroendocrine markers (synaptophysin, chromogranin A), whereas a weak positivity to PSA, and a negativity to racemase were observed in conventional adenocarcinoma foci. Finally, the presence of large eosinophilic granules in « foamy » cells, and the confirmation of their positivity to neuroendocrine markers led one of us to the diagnosis of PLC/NEC. The present observation is of interest for at least three reasons: the rarity of such prostate cancer (the first observation in the African region, to the best of our knowledge), the importance to distinguish this prostate carcinoma pattern among foamy cells adenocarcinomas, and its not yet undebated histologic grading or potential progression data.

Evaluation of the Antineoplastic Activity of Mitoxantrone–L-Carnitine Combination Therapy on an Experimental Solid Form of Ehrlich Tumor in Mice

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We have commenced a series of experiments to evaluate the effect of carnitine derivatives on the antineoplastic activity of mitoxantrone (MX) on various animal cancers. This report describes the therapeutic effect of MX in combination with L-carnitine (LCAR) on the growth of a solid form of Ehrlich tumor (STE) inoculated into mice. LCAR was administered subcutaneously at doses of either 200 or 100 mg/kg on days 6 and 13 after tumor inoculation, one hour prior the treatment with MX. Mitoxantrone was administered intravenously at doses of 3 or 6 mg/kg. We found that LCAR had no potentiating effect on the efficacy of MX, in terms of either slowing tumor growth or increasing the survival of mice. Nevertheless, therapeutic effects can be assumed at higher doses of both drugs based on values calculated from an index of relative hazards.

MORPHOMOUSE, A Genetic Model to Study and Elucidate Biochemical Pathways of Tumorigenesis and In Vivo Drug Screening

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Cancer is a multigenic disease that results as a consequence of dysregulated biochemical pathways that control cell proliferation and survival. The identification of genes and pathways involved in cellular transformation and tumorigenesis has proven to be critical for devising new therapeutic strategies for early-stage detection and for developing specific targeted therapy. Animal models that can accurately recapitulate the normal manifestation of cancer in humans serve as powerful tools for identifying genetic biomarkers and drug targets associated with early-stage cancer for diagnosis and serve as models to evaluate the in vivo activity of drugs capable of suppressing and eradicating growth of malignant cells. We have developed a novel transgenic mouse model of cancer called the MORPHOMOUSE. The MORPHOMOUSE is a transgenic C57BL/6 mouse that expresses a dominant negative allele of a mismatch repair (MMR) gene, called morphogene. Morphogene is an engineered allelic variant of the human PMS2 MMR gene that was identified in kindreds affected

with Turcot's syndrome, a disorder characterized by the concurrence of a primary brain tumor and multiple colorectal adenomas1. Morphogene has been found to act as a dominant negative inhibitor of MMR leading to genetic instability (GIN), a hallmark of MMR deficiency, in various organs of patients that aberrantly express the morphogene and ultimately a genetic predisposition to cancer2,3. Genetic analysis of MORPHOMICE has confirmed these mice to have GIN in all organs tested similar to what is observed in humans. Interestingly, spontaneous cancers have been observed in these mice. Because MORPHOMICE can faithfully reproduce the human disease, these transgenic mice offer a powerful tool to discover disease or disease stage-specific biomarkers that can facilitate preclinical drug candidate selection and accelerate clinical development by potentially improving patient selection. Furthermore, these mice offer the advantage of developing methods for early assessment of drug efficacy in a clinical setting. Moreover, these mice are useful to assess the in vivo effects of drug candidates that can suppress GIN in cells undergoing mutagenesis, a feature that is found in a substantial fraction of all human cancers4. The use of the MORPHOMOUSE platform offers a new opportunity to follow the cellular and molecular progression of genetic event in pre-malignant and malignant cells in vivo and opportunities to develop innovative methods for target discovery and in vivo drug screening and validation. References 1. Hamilton SR, Liu B, Parsons RE, Papadopoulos N, Jen J, Powell SM, Krush AJ, Berk T, Cohen Z, Tetu B, et al. The molecular basis of Turcot's syndrome. N Engl J Med. 1995 Mar 30;332(13):839-47. 2. Parsons R, Li GM, Longley M, Modrich P, Liu B, Berk T, Hamilton SR, Kinzler KW, Vogelstein B. Mismatch repair deficiency in phenotypically normal human cells. Science 1995. 268:738-40. 3. Nicolaides NC, Littman SJ, Modrich P, Kinzler KW, Vogelstein B. A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype. Mol Cell Biol. 1998. 18:1635-41 4. Kabbarah O, Mallon MA, Pfeifer JD, Edelmann W, Kucherlapati R, Goodfellow PJ. A panel of repeat markers for detection of microsatellite instability in murine tumors. Mol Carcinog. 2003 Dec;38(4):155-9

Revolution Technology™ for the Generation of Cell Lines to Discover Phenotypic Pathways and Cells Useful for High Throughput Drug Development

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Mismatch repair (MMR) is a conserved DNA repair process that all organisms use to ensure a high degree of fidelity upon DNA replication. In mammals, cells with defective MMR exhibit an elevated degree of genetic hypermutation in genes that lead to transformation1. Revolution Technology

is a proprietary process employing small molecules and polypeptides that can reversibly regulate MMR in target cells to yield genetically evolved sibs exhibiting phenotypes of interest2. Evolved cells can be used for delineating pathways associated with disease-specific phenotypes by comparative analysis of the parental and evolved cells via standard genomics-based approaches, and for screening therapeutic compounds capable of suppressing defective MMR-mediated hypermutation. Applications of Revolution Technology include generation of drug-resistant/susceptible cell lines; host cells with enhanced/altered signal transduction pathways and/or cells with altered growth properties (nutrient deprivation, cellular adhesion, altered proliferation rates). There are several advantages of Revolution over in vitro gene manipulation techniques and chemical mutagenesis. In general, gene mutagenesis relies on determining which gene is critical for the desired trait, in vitro manipulation and re-introduction into cells to validate a trait of interest. This tedious process depends to a great extent on choosing the correct combination of gene(s) to match the trait at the outset, and is impractical if a phenotype is multi-genetic. In contrast, because Revolution works in vivo, it is possible to evolve the entire genome of the cell resulting in complex mutagenesis at multiple loci to derive a specific phenotype identified by whole cell screening. The net effect is to "accelerate" the natural evolution process in vivo to derive cells with specifically tailored traits. The process also has benefits over standard mutagenesis techniques because MMR results in random non-biased mutations incorporated by DNA polymerase during DNA synthesis and is not toxic to cells thereby resulting in a wider array of evolved sibs with genetic modifications that may exhibit a desired phenotype. Revolution applications have resulted in the generation of cells with single gene mutations and cells exhibiting multi-genetic traits as a result of mutations in multiple loci within the same cell. Cells derived via Revolution can also be combined with proprietary bioluminescent reporter vectors that monitor for defective MMR mediated hypermutation in a high throughput screening format to identify compounds capable of suppressing hypermutability and cancer. The technology has been applied to mammalian and microbial cells to develop variant strains suitable for the development of disease models, biological-based therapeutics and target discovery. References 1. Goel A, Arnold CN, Niedzwiecki D, Chang DK, Ricciardiello L, Carethers JM, Dowell JM, Wasserman L, Compton C, Mayer RJ, Bertagnolli MM, and Boland CR. Characterization of sporadic colon cancer by patterns of genomic instability. Cancer Res. 63:1608-1614, 2003. 2. Nicolaides, NC, Ebel, W, Kline, JB, Chao, Q, Routhier, E, Sass, PM, and Grasso, L. Morphogenics as a Tool for Drug Discovery. Ann. N.Y. Acad. Sci. 1059:1–11, 2005.

Detection of Oral Precancers and Cancers using HPLC-LIF Spectroscopy

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Detection of oral cancers in the early asymptomatic stages dramatically improves cure rates and quality of life by minimising debilitating treatments. One of the challenges for the health professional is early detection and management of this condition. Presently histomorphological analysis has remained the gold standard in the diagnosis, because it has been seen to be the most conclusive of all other diagnostic techniques. However this technique is invasive, subject to human error and work well after the disease has progressed beyond the early stages. The High Performance Liquid Chromatography –Laser Induced Fluorescence Spectroscopy (HPLC-LIF spectroscopy) experimental set up can detect micro amounts of bio-molecules in complex multicomponent physiological samples like human saliva and serum. This system can detect precancers like leukoplakia, lichen planus, oral submucous fibrosis and oral cancer from serum and whole expectorated saliva of individuals.

Chmp1, a Strabismus Binding Protein, Plays Multiple Roles in Vertebrate, Convergent Extension Movements, and Tumor Formation

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Strabismus (Stbm) modulates convergent-extension movements during gastrulation in vertebrates as a component of the Planar Cell Polarity (PCP) pathway. Chromatin modifying protein 1 (Chmp1) was identified as a Stbm binding protein from a yeast two-hybrid screen. Even though Chmp1 protein is highly conserved throughout evolution, the functions of Chmp1 in vertebrate development as well as its signaling activities has been barely investigated. Here, we demonstrated that Chmp1 was recruited to the membrane where it associated with Stbm to regulate convergent extension movements in zebrafish. Similar to Stbm. Chmp1 induced AP-1 reporter activity and phosphorylation of c-Jun, a downstream target of the PCP pathway. In addition, Chmp1 induced an early embryonic neoplasm in zebrafish when its expression was mis-regulated. In NIH 3T3 cells, Chmp1 induced growth inhibition when over-expressed. However, when Chmp1 was knocked-

down by siRNA, NIH 3T3 cells gained the ability of anchorage-independent growth, a phenotype of transformed cells. Chmp1 mRNA was specifically reduced in various pancreatic tumor tissues compared to corresponding normal tissues from the same patient. Examination of human pancreatic tumor array showed absence of Chmp1 protein expression in the acinar cells. Taken together, our results indicate that Chmp1 regulates convergent extension movements via the PCP pathway and may also play a role as a tumor suppressor in certain human tissues such as pancreas.

Effects of Ecdysterone on Insulin Sensitivity and Glucose Metabolism in Insulin-resistant Cell Model

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Objective: To investigate the effects of ecdysterone on insulin sensitivity and glucose metabolism in the insulin resistant HepG2 cell model induced by high concentration of insulin.
Methods: The insulin-stimulated glucose incorporation rate was determined with 3H-D-glucose uptake test which was used to estimate insulin sensitivity of HepG2 cell, and glucose consumption, the amounts of glucose disappeared from the culture medium of HepG2 cells within 24 hours were determined.
Results: the incubation of insulin resistance cells with ecdysterone 1×10-6~10-4M could significantly increase the glucose uptake and glucose consumption of the cells compared with that of control cells($P<0.01$). The glucose uptake and glucose consumption of insulin resistance cells were not of difference in the insulin resistance cells with ecdysterone and pioglitazone ($P>0.05$).
Conclusion: Ecdysterone could improve the insulin sensitivity in the cell model and might attenuate the aggression of insulin resistance. The effect to improve the insulin sensitivity with ecdysterone was similar to that with pioglitazone.

Remarkably Similar Patterns of Immunoglobulin Variable Gene Rearrangement in Human Cancers of Different Tissue Origin

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Immunoglobulin (Ig) gene expression normally associates with lineage fidelity in B lymphocytes and plasma cells, but recent reports have shown that numerous epithelial cancer cells and hyperplastic epithelial cells can also express Ig. In this study, we investigated the expression profiles of IgG, IgM, and Igκ in multiple types of non-hematopoietic tumors and analyzed the tumor-derived Ig gene repertoire of heavy chain (VHγDJHγ, VHμDJHμ) and light chain genes (VκJκ) from microdissected cancer cells in different types of

carcinomas. Igs were widely expressed in non-hematopoietic tumors, and we restricted 11 patterns of clonally related VH sequences with identical VDJ recombination and junctions. Thus, the non-hematopoietic tumor repertoire displayed several dominant V (D) J patterns and oligoclone characteristics. Unexpectedly, all 35 of the analyzed Vκ genes displayed a unique sequence of Vκ and Jκ recombination (Vκ4–1/Jκ3) with identical junctions. More important, the cancer-derived IgG and Igκ genes displayed significant hypermutation. The wide expression of oligoclone Igs in non-hematopoietic tumors and the specific sequence characteristics make possible a new field of investigation into the structure and function of non–hematopoietic-derived Igs. In addition, these findings may have important implications for studies of the clonal origin of cancers and in cancer diagnosis and cancer biotherapy.

Chronotherapy by Ribozyme Targeted to Telomerase in Nude Mice with a Transplanted Tumor: Model for Human Hepatic Cancer

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Earlier, we showed that DNA synthesis (S) in a hepatic tumor was characterized by a statistically significant circadian rhythm, as was telomerase activity. The latter was synchronized with S, peaking around 21 and reaching a trough around 09 hours after light onset (HALO) on a regimen of 12 hours of light alternating with 12 hours of darkness (LD12:12). Herein, we investigate therapeutic outcomes, using telomerase inhibitors to treat cancer at the presumably most and least opportune circadian stages. Twenty-four BALB/C nude mice were synchronized to an LD12:12 regimen for 4 weeks. Hepatic cancer cells (SMMC-7721) were implanted into both flanks of each mouse. Two weeks after transplantation, the hTERT-5'RZ was used to treat the hepatic cancer transplanted into the nude mice, the injection times being either 9 or 21 HALO. The tumor inhibition ratio of mice treated at 21 HALO (66%) was statistically significantly higher than that of mice treated at 9 HALO (50%). Injection of ribozyme targeted to telomerase during the tumor's DNA synthesis is associated with a better inhibition of tumor growth and a better therapeutic outcome. The optimal compromise between chronopharmacokinetics and the chronopharmacodynamics remains to be solved with denser than 2-timepoint sampling.

Molecular Mechanisms of HIF1a Expression in Chronic Hypoxia

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The transcription factor hypoxia-inducible factor 1 (HIF1) is a 'master

switch' governing oxygen response. In response to hypoxia, HIF1a protein accumulates intracellularly and leads to the activation of hundreds of genes involved in cell proliferation, metabolism and angiogenesis. Upregulation of HIF1 is associated with inflammation, poor prognosis in many cancers, and decreased susceptibility of tumors to radiotherapy. Enhanced understanding of mechanisms underlying the HIF1 pathway may lead to new in vivo therapeutic strategies – a goal of this molecular-detailed, HIF1 computational research. We developed a model of the intracellular HIF1 pathway in conditions of chronic hypoxia, such as found in tumors. The model, a set of biochemical reactions, incorporates HIF1a hydroxylation by prolyl hydroxlyases (PHDs); PHD binding to its cofactors; HIF1a degradation; PHD synthesis as a function of oxygen; HIF1a synthesis and HIF1a hydroxylation inhibition by the reaction by-product succinate. Input is cellular oxygen level, and output is HIF1a levels in the nucleus in relation to necessary intermediate reactions. Results of computer simulations show temporally how HIF1a degradation and accumulation rates vary with autocrine feedback from the compounds HIF1a, PHD and succinate. The model predicts the half-life of HIF1a during chronic hypoxia and demonstrates a potential mechanism for cellular adaptation to moderate chronic hypoxia by a balance of PHD and HIF1a synthesis, allowing a return to basal levels of HIF1a. Results are contrasted to the HIF1 response to acute hypoxic exposure. Results offer insight into how a tumor survives in its variably hypoxic environment, and the detailed mechanisms of the model should be useful in developing therapies to regulate HIF1a expression.

FRA-1 Proto-Oncogene Promotes Human Lung Epithelial Cell Growth and Invasion by Inducing MMP9- and EGFR-activated Signaling

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FRA-1 forms AP-1 complexes in association with members of the JUN family and drives gene transcription. FRA-1 has been implicated in the development of airway squamous metaplasia, and is frequently overexpressed in squamous cell carcinomas of esophagus and stomach. We and others have shown a high level of persistent induction of FRA-1 by carcinogens, cigarette smoke and asbestos in pulmonary epithelial cells. However, the exact mechanisms of FRA-1-initated growth, motility and invasion of lung cancer cells are poorly understood. To identify these mechanisms, we have stably overexpressed this transcription factor in a human lung alveolar cancer cell line,

A549 and non-malignant human bronchial epithelial cell line, BEAS-2B. We show that ectopic FRA-1 greatly enhances the rate of proliferation, motility and invasion of both A549 and BEAS-2B cells. In athymic nude mice, injection of A549 cells stably expressing FRA-1 greatly enhanced lung tumor formation, as compared to empty-vector bearing A549 cells. To understand the FRA-1 regulated mechanisms involved in this processes, we have performed in vitro studies using A549 cells. Gelatin zymogram analyses revealed that FRA-1 specifically enhances matrix metalloproteinase-9 (MMP-9) activation by binding to the MMP-9 gene promoter and stimulating the transcription. An MMP-9-specific inhibitor significantly attenuated FRA-1-stimulated cell motility and invasion, supporting a role for MMP-9 in regulating the aggressive growth phenotype of A549 cells.

Nerve Sheath Tumor Resistance QTLs 1 and 2 Increase Tumor Resistance by Different Mechanisms: Using Chromosome Substitution Strains to Dissect Tumor Resistance

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The purpose of this study is to identify genes responsible for variable susceptibility to tumors in neurofibromatosis type 1 (NF1) using a mouse model. NF1 is a familial disease of the nervous system with predisposition to cancer. We are using a mouse model of NF1 (Nf1-/+;p53-/+cis mice) to understand the role of modifier genes in the malignancies associated with NF1. Nf1-/+;p53-/+cis mice develop many of the malignancies associated with NF1, in particular secondary glioblastoma and peripheral nerve sheath tumors (GEM PNSTs). We have found imprinting effects linked to chromosome 11 and strain-specific modifiers that affect the incidence of these tumors, and present data here that this imprinting effect interacts with the p53 mutation, rather than Nf1. The effect of imprinting on chromosome 11 has opposite parental effects on glioblastomas and GEM PNSTs. We recently analyzed the genetic interaction between an imprinted locus on mouse chromosome 11 and the modifier loci Nstr1 on mouse chromosome 19 and Nstr2 on mouse chromosome 15. In this current study we have extended these findings using B6.A chromosome substitution strain to examine the effect of Nstr1 and Nstr2 in isolation on GEM PNSTs. Chromosome substitution strains carrying the A/J chromosome 15 or 19 on a C57BL/6J background were crossed to the Nf1-/+;p53-/+cis mouse model on a C57BL/6J background to generate F1 mice mutant for Nf1-/+;p53-/+cis and carrying one allele of A/J on either chromosome 15 or 19. Differential effects on tumor latency and tumor

penetrance are seen depending on whether the A/J chromosome 15 or 19 is present, suggesting that Nstr1 and Nstr2 act in different genetic pathways. These data confirm the location of Nstr1 on chromosome 19 and Nstr2 on chromosome 15 and further demonstrate that modifier genes affect tumorigenesis under very specific conditions.

Over-expressing RhoC Up-regulates Muc18 Expression in Primary Human Melanoma Cell Line WM35

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Over-expression of RhoC in various human cancers has been correlated with the metastatic tumors and poor prognosis of cancer patients, demonstrating that RhoC plays an important role in tumor metastasis. Cell surface glycoprotein MUC18, a member of the immunoglobulin superfamily and homologous to several cell adhesion molecules, is associated with tumor progression and the development of metastasis in human malignant melanoma. In metastatic melanoma cells, loss of activator protein (AP)-2 expression has lead to over-expression of MCAM/MUC18. We have shown that ectopic expression of RhoC in a radial phase human melanoma cell line, WM35, resulted in its increased invasion ability in vitro. In this report, we investigated whether over-expression of RhoC in WM35 altered Muc18 expression through AP-2. Affymatrix microarray analysis with these cells showed that over-expression of andαand down-regulated AP-2 ,RhoC in WM35 cells up-regulated Muc18 Further RT-PCR analysis validated these results. In addition, RT-PCR .γAP-2 analysis also showed that inhibition of RhoC with C3 transferase treatment reversed the effects of over-expressing RhoC in these cells, indicating that mRNA.γ and AP-2αRhoC indeed up-regulated Mcu18 and down-regulated AP-2 Preliminary Western blot analysis suggested that RhoC up-regulated Muc18 at protein levels. These results suggest that RhoC promotes melanoma metastasis in part through down-regulation of AP2 and up-regulation of MUC-18 gene expression.

Involvement of NF- O(6)-Methylguanine-DNA-methyltransferase Gene Transcription: Possible New B Mediates Tumor Chemoresistance

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O(6)-methylguanine-DNA-methyltransferase (MGMT) and nuclear

factor kappa-B (NF-kB) are two key effectors associated with the development of resistance to alkylating agent-based chemotherapy. This prompted us to hypothesize that NF-kB might be involved in MGMT regulation. Consistent with this hypothesis, we have found two putative NF-kB binding sites within the MGMT promoter region, and have demonstrated by an electrophoretic mobility shift assay a specific and direct interaction of NF-kB at each of these sites. Transfection of HEK293 cells with the NF-kB subunit p65 induces a 90 fold increase in MGMT expression, while addition of the NF-kB super repressor DNIkB completely abrogates this induction. We also found a significant correlation between the extent of NF-kB activation and MGMT expression in both glioma cell lines and human glial tumors. Furthermore, the extent of NF-kB activation in gliomas correlated to MGMT expression, regardless of MGMT promoter methylation status. These findings are of potential clinical significance as we show that cell lines with either a forced expression of p65 or a high constitutive activity of NF-kB are less sensitive to nitrosourea (e.g. BCNU) treatment regardless of MGMT promoter methylation status. The findings of our study strongly suggest that NF-kB plays a major role in MGMT regulation, and may shed light on a novel role of NF-kB in the regulation of DNA damage repair mechanism, and acquisition of chemoresistance.

Syk Kinase Modulates the Malignancy of Pancreatic Ductal Adenocarcinoma Cells and Regulates the Expression of the Malignancy-Associated Antigen CD171

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Pancreatic ductal adenocarcinoma (PDAC) has one of the lowest survival rates of any cancer. We have identified the hematopoietic tyrosine kinase syk as a marker of differentiation/tumor suppressor in PDAC. Syk is uniformly expressed by normal pancreatic ductal epithelial cells, as well as low grade PanIN's. Syk expression is maintained in low grade PDAC tumor cells (G1,G2), irrespective of invasive capability, however syk is absent from high grade PanIN's and PDAC (G3), and syk expression correlates positively with patient survival, suggesting that syk is a tumor suppressor and mediator of the malignant phenotype of PDAC cells. In support of this contention, stable ectopic expression of syk in G3 Panc-1 cells resulted in downregulation of CD171, whose expression is restricted to only the most poorly-differentiated PDAC cells, and which we have previously shown to mediate a malignant phenotype in other cells. Reciprocally, suppression of syk expression in G2 BxPC-3 cells and immortalized normal pancreatic ductal epithelial cells results in de novo CD171 expression. Neither of these effects are due to changes in the steady-state levels of CD171 transcript, suggesting that syk-

dependent regulation of CD171 is post-transcriptional in these cells. Phenotypically, Panc-1 cells expressing syk are growth retarded in vitro, and demonstrate reduced growth and angiogenesis in nude mice. Importantly, these cells exhibit enhanced sensitivity to both radiation and gemcitabine, the chemotherapeutic agent of choice in PDAC. Conversely, BxPC-3 cells stably expressing a dominant-negative form of syk exhibit elevated growth rates and enhanced resistance to both gemcitabine and radiation. Based on these data, we propose that syk is a tumor suppressor and regulator of PDAC malignancy with potential diagnostic/prognostic or potentially therapeutic utility.

p63 Induces Downregulation of Sirt1 and an Accelerated Aging Phenotype in the Skin

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p63 is a homolog of the p53 tumor suppressor gene and plays a unique role in human cancer. In contrast to p53, p63 is rarely mutated in human cancers. However, most tumors maintain p63 expression, coherent with p63 performing a pro-proliferating reaction or a counter reaction to proliferation. p63 uses two promoters and alternative splicing to create different isoforms, with an amino-terminal transactivation domain (TAp63) and without(ΔNp63). Each isoform reacts differently. ΔNp63 isoform is thought to have a dominant negative effect against the proliferating potential of the TAp63 isoform. To examine the dominant negative effect of ΔNp63, we created transgenic mice that overexpress the ΔNp63α isoform in the skin. These mice exhibited an accelerated aging phenotype in the skin characterized by striking wound healing defects, decreased skin thickness, decreased subcutaneous fat tissue, hair loss, and decreased cell proliferation. The accelerated skin aging was accompanied by a dramatic decrease in longevity of the mice. We found that aging in ΔNp63α transgenic mice and other mouse models correlated with levels of Sirt1, a mammalian SIR2 orthologue thought to extend the lifespan in lower species. Moreover, increased ΔNp63α expression induced cellular senescence that was rescued by Sirt1. Our data suggest that ΔNp63α levels may affect aging in mammals, at least in part, through regulation of Sirt1.

Antitumor beta-Lactam Derivatives with Serine Protease Inhibiting Properties

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Background: The discovery of potent elastase inhibiting properties for structurally modified beta-lactams in 1986 terminated their antibacterial “monopoly” and opened new

perspectives in the treatment of diseases based on reducing in human organism the excessive activity of various serine or cysteine proteases: inflammation, immunological, respiratory, cardiovascular disorders, cancer, etc. The appropriate investigations carried out in our laboratory have led to the discovery of beta-lactams with potent antitumor properties.

Methods: A series of beta-lactam derivatives on the basis of cephem, penam, and 2-azetidinone templates were synthesized and subjected to cytotoxic screening in vitro using standard MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma) cell lines the same as normal NIH3T3 (Swiss Albino mouse fibroblasts) and BHK-21 (baby hamster kidney) cell lines. The inhibiting effect for most active compounds was proved in vivo towards transplanted sarcoma and mouse hepatoma tumors.

Results: The analysis of SAR for antitumor beta-lactams revealed their remarkable resemblance with anti-inflammatory cephalosporin derivatives acting as human leukocyte elastase (HLE) inhibitors. It enabled to conclude that on the molecular level both activities were represented by a similar protease inhibition mechanism. Accordingly, specific elastases promoting cancer development and proliferation could be regarded as a molecular target for the investigated beta-lactam derivatives.

Conclusions: The results obtained make the search for new antitumor compounds among beta-lactam derivatives rather promising. The postulated mechanism of their action, based on the inhibition of specific serine proteases responsible for the tumor growth and proliferation, distinguishes them from the prior art classes of anticancer drugs, the effectiveness of which is endangered by the tumor resistance phenomenon.

P-gp Related Drug Resistance in Human Hepatoblastoma

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Background: Hepatoblastoma (HB) is the most common primary malignant liver tumor in children. Despite improved overall survival rates, treatment results of advanced tumors are, however, still far from being satisfying. Multidrug resistance has been identified as a major factor responsible for the bad prognosis of children suffering from advanced staged HB. The aim of our study was to identify the role of P-glycoprotein (P-gp) in the development of drug resistance in HB.

Materials and Methods: P-gp expression levels were assessed in respected specimen from HB, in cell cultures and in HB after xenotransplantation into nude mice. Tumor cells and animals were treated with either cisplatin or adriamycin. Additionally, the effects of chemosensitizers (verapamila and PSC 833) against P-gp on the chemotherapy were determined in vitro and in vivo. Results: P-gp and MDR1 gene expression levels in the tumor specimen were enhanced in correlation to the

applied courses of chemotherapy: untreated tumors revealed the lowest expression, tumors after standard chemotherapy higher presented higher levels, and tumor relapses showed the highest levels of P-gp expression. Chemosensitisers significantly improved treatment results of cisplatin and doxorubicin in two HB cell lines in vitro (cell viability) and in vivo (animal survival, tumor volumes and serum-alpha feto protein levels).
Conclusions: P-gp plays an important role in the development of multi drug resistance in HB. The P-gp related drug resistance could be reduced successfully through chemosensitizers. This approach might offer an important contribution to the treatment of advanced HB.

Cancer Patient Education

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There are four major aspects to good cancer care. First and foremost is reduction of tumor burden (surgery, chemotherapy and radiotherapy). More subtle, but also vital, are immune system enhancement, energy repletion, and will-to-live. We are not adequately serving the patients' three latter needs. These latter principles involve patient education which is less science-oriented than reduction of tumor burden, including "complementary" and spiritual disciplines. And in these areas the patient has the more active role than the physician; but patients need guidance in choosing these modalities. When competent immune systems and adequate energy stores are in place, and there is a will to live, our assumption is that the patient may avoid the fatigue and deterioration which result in death. There is no guaranteed cure for cancer, only long-term remission. Since cancer is such a threat, and more complex than diabetes, our education of cancer patients must be as thorough, vigorous and even more broad than diabetic education. And the provision of this training will, in itself, counter the "loss of control" and despair which characterize facing "the big C". We accomplish this program through any one of several formats, but usually involving two to three day seminars or retreats having both lectures and demonstrations. The curriculum is intensive and inclusive, drawing from up-to-date scientific fields such as psychoneuroimmunology as well as credible complementary sources and conventional medicine. Speakers present papers on medical oncology, immunology, nutrition, massage, exercise therapy, yoga, meditation, and attitudinal healing. At present, a panel of eight speakers has been assembled on Oahu to present this program. Richard S. Weeder, M.D. FACS For Aloha Cancer Education Institute

Functional Genomics of Chemosensitivity Exposes Deviant Cancer Cell Regulatory Systems

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Widespread evidence suggests that a unifying principle governing the molecular pathology of cancer is the co-dependent aberrant regulation of core machinery driving proliferation and suppressing apoptosis. Anomalous proteins engaged to support this tumorigenic regulatory environment likely represent optimal intervention targets in a heterogenous population of cancer cells. The advent of RNAi-based functional genomics provides the opportunity to derive unbiased comprehensive collections of validated gene targets supporting critical biological systems outside the framework of preconceived notions of mechanistic relationships. Here, we have combined a high throughput cell-based one-well/one-gene screening platform with an arrayed genome-wide synthetic siRNA library for systematic interrogation of the molecular underpinnings of cancer cell chemoresponsiveness. A human non small cell lung cancer line was employed for a paclitaxel-dependent synthetic lethal screen designed to identify gene targets that specifically reduce cell viability in the presence of otherwise sub-lethal paclitaxel concentrations. Using a stringent objective statistical we isolated a panel of 87 genes that represent major fulcrums of the cancer cell autonomous response to abrogation of microtubule dynamics. Importantly, a number of these targets sensitize lung cancer cells to paclitaxel concentrations 1000-fold lower than otherwise required for a significant response, and reveal novel mechanistic relationships between cancer-associated aberrant gene expression programs and the basic cellular machinery required for robust mitotic progression.

Feasibility of Immunohistochemical Study of Tumor Markers (XIAP, Histone H1 and p63) in FNA Cellblock Samples from Head and Neck Squamous Cell Carcinoma

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Background: One of the major handicaps in contemporary clinical oncology is the inability to predict the responsiveness of any individual's malignancy to specific therapies. The purpose of this study was to test the feasibility of immunocytochemically detecting markers that may be affected by therapy or predictive of therapeutic responsiveness, including phospho-histone H1 (p-HH1) and X-linked inhibitor of apoptosis (XIAP) in small samples obtained via a minimally invasive fine needle aspiration (FNA) biopsy procedure, thus improving therapeutic monitoring. p63, a squamous stem cell regulatory protein was also examined. These 3 markers were studied in FNA cell block samples of head and neck squamous cell carcinoma (HNSCC).

Materials and methods: Twenty-eight (28) alcohol-fixed formalin-post-fixed paraffin embedded cell-block samples from FNAs of patients with primary and metastatic HNSCC were subjected to citrate based antigen retrieval then incubated with monoclonal anti-XIAP (# 610763, BD Biosciences, San Jose, USA) 1:250, 4 C for 72 hrs and developed using EnVision-Plus reagents (Dako) and diaminobenzidine as chromagen; and with monoclonal anti-p63 (4A4, Santa Cruz), 1: 4000 and anti-p-HH1, 1:10 in PBS with 0.1% bovine serum albumin and 5% non-fat dry milk in room temperature for 16 hrs except that 1M NaCl was added to PBS for p-HH1. Granular or heterogeneous cytoplasmic staining for XIAP and nuclear staining for p63 and p-HH1 were considered positive.
Results: Among the 28 cases studied, the overall positive rates for XIAP, p-HH1 and p63 were 60.7%, 96.4% and 92.8%, respectively. The staining intensity for XIAP: + 70.6%, ++ 23.5%, +++ 0% and ++++ 5.9%; for p-HH1: +48.1%, ++ 11.1%, +++37.0% and ++++ 3.7% and for p63: + 11.5%, ++ 23.1%, +++ 53.9% and ++++ 11.5.0%. The expression of nuclear markers p-HH1 and p63 appeared to be higher and stronger than that of XIAP in HNSCC.
Conclusion: This study demonstrated the feasibility of monitoring expression of three valuable tumor markers using FNA samples. p-HH1 and XIAP may be useful for monitoring actions of cyclin-dependent kinase inhibitors, and XIAP-lowering and/or apoptosis inducing drugs, respectively. Future studies will focus on the impact of these and other therapies upon these staining profiles.

2-Deoxyglucose Induces a Metabolic Switch to Mitochondrial Oxidative Energy Metabolism Resulting in Sensitization to Apoptosis of Glioblastoma Cells

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Many tumor cells display high rates of glucose utilization, a phenomenon known as the Warburg effect. This glucose dependency in malignant cancer cells has been shown to be driven by multiple oncogenic pathways that affect cellular energy metabolism. Impaired mitochondria respiration in cancer cells, as Warburg proposed in the 1920s, has recently been demonstrated using mitochondrial biomarkers in a number of human tumors. This impairment may be a mechanism of tumor cell resistance to apoptosis. In fact, compounds that target cancer metabolism, such as the hexokinase inhibitor, 2-Deoxyglucose (2-DG), are now in clinical trials. By measuring in real-time, oxidative and glycolytic energy metabolism, we found that 2-DG reversed the Warburg effect in the glioblastoma cell line, LN-18. That is, it promoted oxidative phosphorylation as measured by an increased oxygen consumption rate (OCR) per cell as well as a reduced rate of glycolysis measured by a decreased extracellular acidification rate (ECAR) per cell. The metabolic shift towards oxidative ATP production was confirmed by an observed increase in the sensitivity of cellular ATP levels to the

ATP synthase inhibitor, oligomycin, and conversely a decreased sensitivity to the LDH inhibitor, oxamate. Interestingly, apoptosis induced by staurosporine (STS) was dramatically increased in 2-DG treated cells compared to the control untreated cells. Our data suggest that changes in metabolic rates may be one of the earliest indicators of apoptosis. In conclusion, we propose that 2-DG inhibition of glycolysis drives LN-18 cells to a more oxidative state accompanied by growth suppression and sensitization to STS induced apoptosis. We believe that these results support the increasing interest in targeting metabolism for cancer therapy and how our approach can aid in the identification of anti-cancer compounds that exploit aberrant glucose metabolism.

Studies on Anticarcinogenic Activity of Sulforaphane in Broccoli

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A lot of literatures demonstrate that sulforaphane (1-isothiocyanato-4-(methylsulfinyl)-butane) is the main component with anticarcinogenic activity in broccoli. Anticarcinogenic studies indicate that broccoli has an obvious effect on lung cancer, esophagus cancer, forestomach cancer, and its mechanism is to induce the generation of Phase Ⅱ (detoxifying carcinogenic factor) enzymes (such as GST, NAD(PH)-quinone reductase), further affect the anticarcigennic system of its own to modulate its metabolism: (1) stop the metabolic course that is needed by carcinogens, stop the carcinogens and their metabalities entering the targeted cells, and also eliminate the carcinogens; inhibit the mitosis and reproduction of tumor cells, block the circulation course; promote the secretion of protein that can kill tumor cells; stop the carcinogens from inducing cancer. (2) sulforaphane may have some effects on the carcinogens at the very beginning, cause gene mutation, besides there are other inhibitive enzyme which have the ability to inhibit cancer, and can degrade the toxins. Meanwhile, sulforaphane can inhibit the generation of Phase Ⅰ enzyme, decrease the active level of P450 and inhibit its catalytic activity, finally affects the generation of carcinogens, accelerate the process of excreting by destroying the active center of cancerogenic factor or bind its internal ligand, exert its antitumor effect. Sulforaphane is the most effective inducement of Phase Ⅱ ever found, it is the metarial founation that broccoli is able to prevent cancer. Keywords Broccoli, sulforaphane, anticarcinogenic activity Acknowledgement: Thanks for the Committee of National Natural Science Foundation of China, by which the project is supported. (NO.30300284, NO.30400352), Natural Science Foundation of Heilongjiang province for postgraduates (YJSCX2005-133HLJ).

Molecularly Targeted Cancer Chemo/Radiosensitization In Vitro and In Vivo by Modulating Apoptotic Pathways

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Chemo/radioresistance markedly impairs the efficacy of cancer therapy and involves anti-apoptotic signal transduction pathways that prevent the treatment-induced cell death. Anti-apoptotic proteins such as Bcl-xL, Bcl-2, Mcl-1, and inhibitors of apoptosis proteins (IAP), are overexpressed in many types of cancers and contribute to tumor initiation, progression and resistance to therapy. They are promising molecular targets for modulating therapeutic resistance of cancer. We recently demonstrated that, (-)-gossypol, a natural product from cottonseed and a potent small molecule inhibitor of both Bcl-2 and Bcl-xL, can radiosensitize prostate cancer cells. In a PC-3 xenograft model in nude mice, orally administered (-)-gossypol achieved a greater efficacy with tumor regression when used in combination with ionizing radiation. Tumors treated with (-)-gossypol plus radiation showed much more increased apoptosis and reduced angiogenesis. A new and more potent Bcl-2 small molecule inhibitor, TW-37, also showed chemosensitizing activity in breast and prostate cancer animal models. We have also tested a series of novel small-molecule IAP-inhibitors. They potently enhanced apoptosis in cancer cells and sensitized cancer cells to TRAIL and radiation. In nude mouse xenograft models, IAP-inhibitor SH-130 potently sensitized the DU-145 tumors to X-ray radiation. Bioluminescence imaging confirmed SH-130 plus radiation resulted in complete tumor regression in 6 out of 10 tumors, comparing 2/10 tumors with radiation alone, and 0/10 tumors in single therapy. Taken together, our results demonstrate that small-molecule inhibitors of anti-apoptotic proteins Bcl-2/Bcl-xL or IAPs can overcome apoptosis-resistance and sensitize human cancers to chemo/radiotherapy. (-)-Gossypol is now in Phase I-II clinical trials. Molecular modulation of anti-apoptotic proteins may improve the outcome of conventional cancer therapy and represents a promising molecularly targeted therapy for human cancer.

Human Calprotectin-Associated Drug Resistance in Gastric Cancer cell Line in Compare to Other Anticancer Drugs

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Calprotectin and etoposide are cytotoxic agents that their anti-tumor effects is governed through suppression of topoisomerase II; a key enzyme in apoptosis. In previous studies, topoisomerase II poisons(intercalators and nonintercalators) that kills cancer cells by different mechanism were shown cross-resistance pattern of classic (P-glycoprotein-mediated) MDR. In the

present study, cytotoxicity effects of calprotectin and anticancer drugs (etoposide , cicplatin and doxorobicin) associated to cyclosporine (inhibiting expression of MDR) and penicillin (enhancing expression of MDR) on AGS cell line were investigated . For these evaluations, AGS cells were exposed to the LC50 concentrations of either calprotectin or anticancer drugs associated to cyclosporine and penicillin, for 48 hours. Cell proliferation was assessed using MTT assay. Our results revealed that combination of both calprotectin and anticancer drug with cyclosporine induce growth inhibition more than toxic agents and combination with penicillin showed vise versa results. Results of this investigation showed that induction of drug resistance with human calprotectin in gastric cancer cell line is similar to the anticancer drugs. Keywords: Human calprotectin; anticancer drugs; AGS; MDR

Analysis of Differential Protein Components in Renal Cell

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Renal cell carcinoma (RCC) is one of the most malignant tumors in urology, and due to its insidious onset, patients frequently have advanced disease at the time of clinical presentation. Furthermore, RCC is also one of the carcinoma mostly resistant to conventional therapies such as radiation and chemotherapeutic drugs. Thus, it is crucial to find special antigen of RCC in order to early detect and be made as tumor vaccine to performe immunotherapy. To identify tumor specific antigen of RCC, we employed proteomic analysis. We prepared proteins from clear cell RCC and the corresponding normal kidney tissues. The expression of proteins was determined by Coomassie stain after two-dimensional polyacrylamide gel electroph-oresis(2D-PAGE). The overall protein expression patterns in the RCC and the normal kidney tissues were quite similar except some areas. Of 36 differentally expressed protein spots, 4 different proteins were identified by MALDI-TOF-TOF mass pectrum (MS). The expression of the following proteins was repressed: L-arginine:glycine amidinotransferase (AT), Betaine-homocysteine S-methyl- transferase (BHMT), Ketohexokinase (KHK) and neuropolypeptide h3.These repressed proteins may involve in the occurrence of RCC.

Elevated Levels of Cell-Free Serum DNA are Associated with Breast Lesions

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Background: The presence of cell-free DNA in plasma and serum opens up opportunities for diagnosis and evaluation of the prognosis of cancer. We analysed cell-free DNA circulating in the serum of patients with both malignant and benign breast lesions by

real-time quantitative PCR to determine whether the finding have diagnostic and prognostic implications.

Methods: Serum samples were obtained from 33 patients with breast cancer and 32 patients with benign breast tumour. 50 women, who were healthy by clinical detection, ultrasound and mammography, were recruited as normal controls. The tumour size, lymph node involvement, distant metastases, histological grade, oestrogen receptor, progesterone receptor and Her-2-Neu expression in tumour tissues were analysed according to standard methods. Circulatory cell-free DNA was extracted from the serum fractions and quantified by real-time quantitative PCR for GAPDH gene.

Results: The mean number of cell-free DNA genome equivalents (GE) in the serum samples drawn from patients with breast cancer, patients with benign breast tumour and normal controls was 41149, 30826 and 13267, respectively. Levels of cell-free DNA in the serum samples from patients with breast cancer and benign breast lesions were significantly higher than in those samples from normal controls (P=0.001 and 0.031 respectively). These findings were not associated with malignant tumour size, lymph node involvement, distant metastasis histological grade, the status of hormone receptors, and expression of Her's-2-Neu gene.

Conclusions: Our results suggest that levels of cell-free DNA in the serum are elevated in both malignant and benign breast lesions. High level of cell free serum DNA could not serve as diagnostic and prognostic marker to distinguish malignant and benign breast tumours.

LAPTM4B, A Novel Oncogene and a Novel Cancer Target (II)

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LAPTM4B is a novel gene (AY057051) that cloned and identified in our lab from hepatocellular carcinoma (HCC). The LAPTM4B is not only overexpressed in (87.3% HCC), but also upregulated in a number of other cancers, including lung cancer, breast cancer, gallbladder cancer, gastric cancer, colon cancer, renal cell carcinoma, bladder cancer, etc., as well as a number of cancer cell lines. The expressions of LAPTM4B either at mRNA or at protein levels both correlate significantly with differentiation and pathological grades of HCC. Importantly, there are two isoforms of LAPTM4B-encoded protein: LAPTM4B-35 and LAPTM4B-24, which play antagonistic roles. In cancer tissues the upregulated protein is LAPTM4B-35 and the ratio of LAPTM4B-35/ LAPTM4B-24 is increased. We found in this study that overexpression of LAPTM4B-35 in human cells caused by transfection induced progression of malignant transformation, including resistance of apoptosis, enhancement of cell proliferation, migration and invasion, serum-independent proliferation, capacity of colony formation in soft agar, as well as enhanced therapeutic resistance, including drug efflux and cancer cell survival. Notably, overexpression of LAPTM4B by

transfection further induced genome instability, including activation of some proto-oncogenes, such as remarkable upregulation of c-Myc, c-Jun and c-Fos, and downregulation of some tumor-suppressor genes, such as p21, p27 and p16. By contrary, all the cellular and molecular alterations associated with overexpression of LAPTM4B are reversely changed by transfection of LAPTM4B-RNAi plasmid. Accordingly, the overexpression of LAPTM4B in human L02 liver cell line caused dramatic tumorigenesis. In conclusion, LAPTM4B is a novel oncogene and it is predicted that the LAPTM4B gene and LAPTM4B-35 protein may be important targets for gene therapy or chemotherapy.

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