KARIN GRUNEBAUM
cancer research foundation

October, 2010 • Volume 7

Founded in 1958

From the Chair

Dear Friends of the Karin Grunebaum Cancer Research Foundation;

Everyone loves a winner, and we are no exception. We are very pleased to tell you that one of our three 2010 - 2011 Faculty Research Fellows, Michael H.A. Roehrl, M.D., Ph.D., has been awarded the Young Investigator Award by the Human Proteome Organization (HUPO). This prestigious award was awarded to Dr. Roehrl at the organization's international meeting in Sydney, Australia. There is more detail about Dr. Roehrl's pioneering work inside the *Newsletter*.

We salute Dr. Roehrl's accomplishment as he joins the long list of Karin Grunebaum Fellows who have distinguished themselves in academic medicine and medical research. The "Focus on Research" section on the Foundation's website (www.grunebaumfoundation.org) highlights their accomplishments. We would really appreciate hearing from other past or present Fellows who have also achieved unique recognition in their field. Please let me know so we can also highlight your accomplishments.

As many of you know, our Foundation is unique in that the Board of Trustees is composed of pre-eminent figures in academic medicine and cancer research, along with members of Karin Grunebaum's immediate family. Thus, we are very proud to announce that Karin's two youngest grand-children (Shawna Wallach and Christopher Kelly) have joined the Board of Trustees. We know that they will superbly complement the outstanding cadre of Board members.

One of the greatest benefits we receive as Trustees (we are all unpaid volunteers) is to annually hear presentations from the current incoming and outgoing Fellows on their research projects. The incoming Fellows tell us of their hypotheses and projects, while the outgoing Fellows tell us of their accomplishments. This is such an enlightening experience, that the Trustees decided to invite all former Fellows to attend these presentations. The presentations are held annually on the

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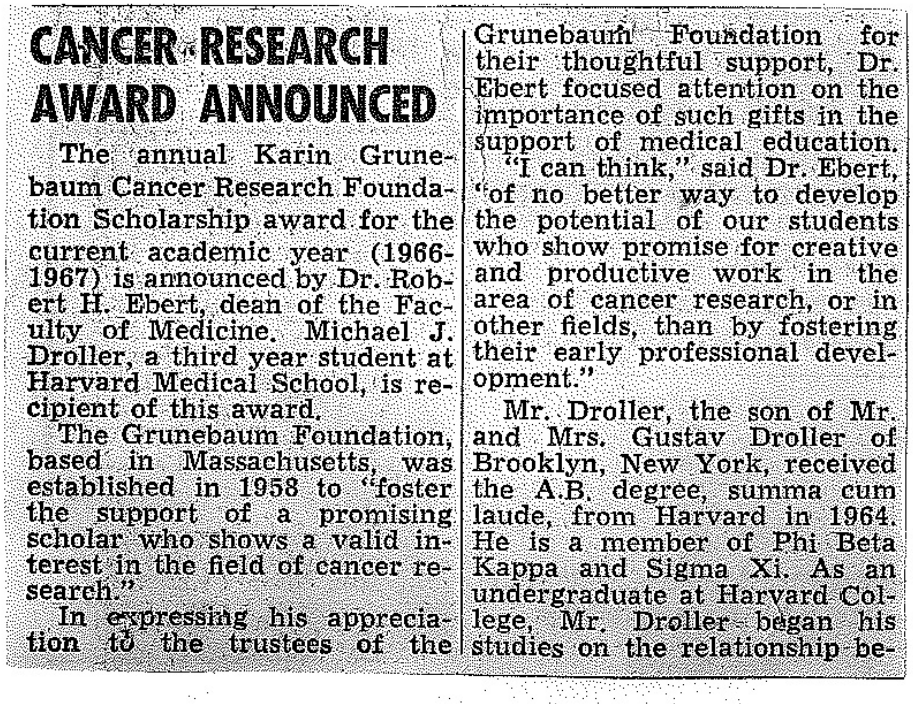
www.grunebaumfoundation.org

From the Archives

Did you know that our very own trustee, Michael J. Droller, M.D.,Chairman Emeritus, was the first recipient of the Karin Grunebaum Cander Research Scholarship?

The following is the newspaper article announcing the award.



CANCER RESEARCH AWARD ANNOUNCED

The annual Karin Grunebaum Cancer Research Foundation Scholarship award for the current academic year (1966-1967) is announced by Dr. Robert H. Ebert, dean of the Faculty of Medicine. Michael J. Droller, a third year student at Harvard Medical School, is recipient of this award.

The Grunebaum Foundation, based in Massachusetts, was established in 1958 to "foster the support of a promising scholar who shows a valid interest in the field of cancer research."

In expressing his appreciation to the trustees of the Grunebaum Foundation for their thoughtful support, Dr. Ebert focused attention on the importance of such gifts in the support of medical education. "I can think," said Dr. Ebert, "of no better way to develop the potential of our students who show promise for creative and productive work in the area of cancer research, or in other fields, than by fostering their early professional development."

Mr. Droller, the son of Mr. and Mrs. Gustav Droller of Brooklyn, New York, received the A.B. degree, summa cum laude, from Harvard in 1964. He is a member of Phi Beta Kappa and Sigma Xi. As an undergraduate at Harvard College, Mr. Droller began his studies on the relationship be-

Dr. Droller is currently the Katherine and Clifford Goldsmith Professor of Urology, Professor of Oncology, Department of Urology, The Mount Sinai Medical Center.

From the Chair *(continued from page one)*

second Friday in June at Boston University School of Medicine. If you would like to attend, please RSVP at least 2 weeks ahead of time. We would love to see you again, and we think you would really enjoy the event.

The Board of Trustees is constantly looking at new avenues to provide support for cancer research. In addition to annually sponsoring first-rate cancer researchers, we are currently looking at ways to also help educate other budding scientists who want to focus their energy specifically on cancer research. We are hopeful that such a "seed" program can also be supported by the Foundation if sufficient additional funds can be raised. Of course, adding to our mandate in these trying economic times requires additional resources to help assure such expansion of our efforts. We sincerely appreciate the donations received during the past year, and we ask each of you to please contribute to our Foundation so that these additional dreams can be realized. Please make your tax deductible contributions by mail or on-line .We believe that when more people are focused on finding a cure for cancer, the sooner that elusive dream can be realized.

Sincerely,

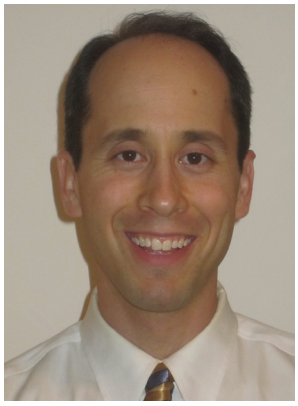
Steven Wallach, Chairperson
(561) 750-7366
E-mail: steven.wallach@grunebaumfoundation.org

From the Fellows

Harvard Medical School

Benjamin Gewurz, M.D., Ph.D. Instructor in Medicine,
Brigham and Women's Hospital, Department of Medicine

Identification of Novel NF-kB Pathway Regulators in Lymphomagenesis



Nuclear Factor-kappa B (NF-kB) is a family of transcription factors that control lymphocyte proliferation, differentiation and survival. Immune receptors activate NF-kB through either or both of two signal transduction cascades, termed 'canonical' and 'non-canonical' pathways. Receptor cytoplasmic domains recruit adaptor molecules that initiate downstream signal transduction events. NF-kB pathways trigger proteasomal degradation of inhibitory proteins, NF-kB nuclear translocation, and activating post-translational modifications of NF-kB complexes. Activated NF-kB drives transcription programs that promote cell growth and that block apoptosis. Multiple negative feedback loops tightly control NF-kB activation and serve to terminate signaling in the absence of persistent stimuli. While NF-kB activity is integral to normal immune system development and function, aberrant NF-kB hyperactivation underlies the growth of numerous lymphomas. Despite intensive study, potentially druggable enzymatic regulators of NF-kB and their importance in specific malignancies remain to be identified.

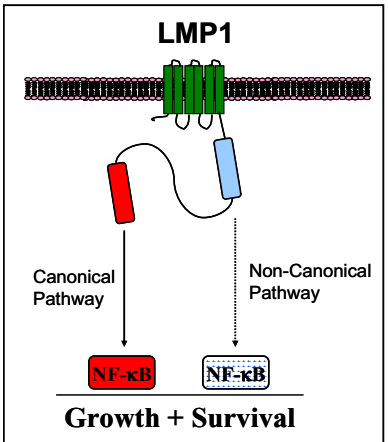


Figure 1. LMP1 stimulates NF-κB through two cytoplasmic signaling domains. The membrane proximal domain triggers non-canonical NF-κB activation, whereas the distal domain stimulates canonical NF-κB.

Gene profiling studies have demonstrated particularly high NF-kB activity levels in multiple aggressive lymphomas: diffuse large B-cell Lymphoma, Hodgkin Disease, primary mediastinal B-cell lymphoma, and multiple myeloma. The genetic basis for their elevated NF-kB activity remains largely unknown, though appears to arise from the mutation of key canonical and non-canonical NF-kB pathway regulators. Notably, NF-kB pathway mutations may contribute to the development of malignancies throughout the body. A recent survey of 3131 human cancer specimens spanning 26 histological types found NF-kB pathway genes to be among the most frequently targeted by somatic copy number alterations. NF-kB activating mutations span several classes of enzymes, including ubiquitin ligases, deubiquitinating enzymes, and kinases. Such mutations appear to collectively amplify activation pathways and diminish negative feedback regulation. Aggressive lymphomas frequently become dependent on constitutive NF-kB activation, and undergo apoptosis upon NF-kB blockade. Unfortunately, there are no therapeutic agents currently available for clinical use to specifically block NF-kB activity. NF-kB pathways therefore contain promising targets for the development of rational chemotherapeutic agents to treat common lymphoid malignancies.

Epstein Barr Virus (EBV) is a ubiquitous herpesvirus that is an important cause of Hodgkin disease and other lymphoproliferative diseases, particularly in the setting of immune suppression. The primary EBV oncoprotein, Latent Membrane Protein 1 (LMP1), mimics NF-kB hyperactivation states present in lymphoid malignancies. LMP1 is expressed in most EBV-associated lymphoproliferative malignancies, including in 40% of Hodgkin lymphoma samples. In a ligand-independent fashion, LMP1 constitutively activates both canonical and non-canonical NF-kB pathways through two independent cytoplasmic tail domains (Figure 1). EBV-transformed cells require persistent NF-kB activation for their growth and survival, and undergo apoptosis upon NF-kB blockade. Small molecule inhibitors of LMP1/NF-kB could therefore potentially inhibit the malignant growth of EBV-infected human malignancies.

My central hypothesis is that EBV-transformed cells and lymphoid malignancies rely on shared cellular signal transduction pathway components to persistently activate NF-kB, the inhibition of which can be utilized to specifically treat human malignancies. To elucidate novel NF-kB pathway components, I have recently completed a human genome-wide loss-of-function siRNA screen to comprehensively identify the LMP1/canonical NF-kB pathway. The screen successfully identified most pathway components, as well as many novel suppressors and enhancers of the LMP1/canonical NF-kB pathway. Hits included numerous enzymes: kinases, phosphatases, ubiquitin ligases, and deubiquitinating enzymes. I have found that 81% of screen hits likewise function in ca-

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Massachusetts General Hospital

Andrew Liss M.D.
Massachusetts General Hospital

Molecular and Functional Characterization of Cancer Stem Cells in Pancreatic Cancer

Last year in the United States more that 42,000 people were diagnosed with pancreatic ductal adenocarcinoma (PDAC) with at least 35,000 people dying from this disease. Currently, surgery provides the only hope for survival. However, 70% of patients are not eligible for surgery since they are diagnosed with locally advanced disease and distant metasteses. While gemcitibine has been the standard of care for PDAC for more than a decade, it has failed to dramatically improve the survival of patients. Less than 5% of patients diagnosed with PDAC survive five years after diagnosis.

Similar to other cancers, PDAC is composed of an assortment of phenotypically distinct tumor cells. Recent cancer research has begun to focus on a subpopulation of tumor cells termed cancer stem cells. Cancer stem cells have the unique capacity for self-renewal, the ability to differentiate into the cells that make up the bulk of the tumor, and efficiently initiate tumor formation in animal models. A number of reports have demonstrated that cancer stem cells are resistant to chemotherapy. The resistance of these cells to traditional chemotherapeutics is consistent with a model in which chemotherapy fails to kill the cancer stem cell population, allowing for tumor recurrence. This model makes cancer stem cells an attractive target for the development of novel therapeutics.

Three populations of cancer stem cells have been reported in PDAC, $CD44^+/CD24^+/ESA^+$, $CD133^+$, and $ALDH^+$ cells. In addition to forming primary orthotopic tumors in mice, a subpopulation of these stem cells ($CD133^+/CXCR4^+$) has metastatic potential. PDAC-derived stem cell populations are resistant to treatment with gemcitibine. Importantly, recent studies have demonstrated that combinatorial treatment of tumors with gemcitibine and inhibitors of stem cell signaling pathways (Shh and mTOR) effectively reduce the tumorgenic potential of these cells in a mouse model.

My experiments will build upon this work to identify novel genes and signaling pathways that are required for the tumorigenesis of PDAC-derived cancer stem cells. The approaches described below will employ a genome-wide RNAi screen specifically targeting the cancer stem cell population of PDAC tumors and a molecular characterization approach to profile the stem cell population compared to its non-tumorigenic counterpart.

Specific Aim 1. Identification of genes required for tumorigenesis of cancer stem cells in PDAC.

A genome-wide shRNA screen will be employed to identify genes whose expression is important for the ability of PDAC-derived cancer stem cells to form tumors in vivo. shRNAs are small RNA molecules that can bind to cellular mRNAs, induce their degradation, and thereby down-regulate gene expression. By utilizing tens of thousands of shRNAs that target nearly every gene in the genome one can evaluate the contribution of a single gene to the biology of a cell in an unbiased manner. Similar screens have been employed in human systems to characterize genes important for cell survival, proliferation, and activated oncogene-dependent growth. These genome-wide screens have proven to be powerful in identifying novel functions and genetic interactions for a variety of genes.

Purified cancer stem cell populations from PDAC tumors will be infected with lentiviruses expressing shRNAs and injected into immunodeficient mice. Half of the injected mice will be maintained under normal growth conditions and the other half treated with gemcitibine. Once tumors have grown, they will be excised and the presence or absence of specific shRNAs will be determined by microarray analysis. Those shRNAs that are reproducibly depleted from the population will be considered anti-tumorigenic. Genes that are targeted by the shRNAs will be the focus of future analysis. We anticipate that many of the depleted shRNAs will be in common between mice treated with and without gemcitibine. However, depleted shRNAs unique to the gemcitibine treated animals may identify novel mechanisms of chemo-resistance in PDAC.

Specific Aim 2. Molecular characterization of PDAC-derived cancer stem cells.

Transcriptome analysis will be simultaneously employed to identify genes that are differentially expressed between the cancer stem cell and non-tumorigenic cell populations. RNA will be isolated from both the cancer stem cell and non-stem cell populations. Global gene expression as well as microRNA analysis will be conducted using GeneChip miRNA and Gene Arrays (Affymetrix). This non-functional approach will allow the identification of genes and pathways that might be critical or aberrant in PDAC derived cancer stem cells. The results of these experiments may also identify genes that, while not functionally important for cancer stem cells, may be amenable to targeted therapeutic approaches.

Boston University Medical School

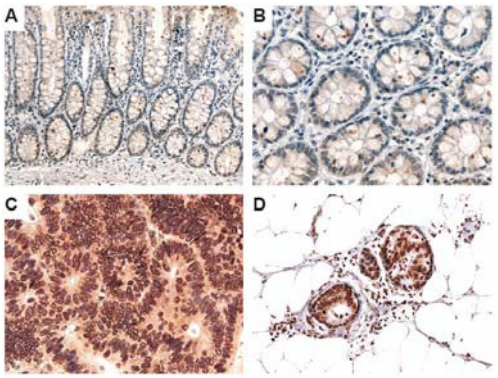
Michael H.A. Roehrl, M.D.

Transformative Pathology and Systems Biology of Cancer

An overarching theme of my research is the experimental investigation of human cancers, specifically colorectal adenocarcinomas. *Colon cancer remains a leading cause of cancer mortality in both men and women.* Yet efficient methods of detection and treatment remain difficult. We are particularly interested in two areas: (1) Molecular characterization of global protein abnormalities in cancer tissues; and (2) isolation and therapy-predictive use of cancer stem cells from primary human tumor tissue retrieved intraoperatively.

1. Proteomic Biomarkers of Colorectal Adenocarcinoma

We have started to investigate the unbiased proteomic composition of human tumor tissues using a combination of 2-D gel electrophoresis, fluorescence labeling, and advanced biophysical techniques (such as high-resolution tandem mass spectrometry) in combination with computational bioinformatics tools. By developing an innovative new subproteome enrichment technology, we were, for the first time, able to interrogate information-enriched tissue proteomes by overcoming the traditional bottlenecks of tissue proteomics, *i.e.*, dynamic range, discoverable proteomic depth, and informative content. Using these newly developed methods, we went on to study clinical samples of colorectal cancer and discovered that PSB7 (proteasome subunit b7), PRDX1 (peroxiredoxin-1), and SRP9 (signal recognition particle 9 kDa protein) are important proteins in colorectal carcinomas, indicating a hypoxia-adaptive tumor response. We demonstrated that all three proteins are produced by the *bona fide* cancer cell compartment, not the surrounding induced stroma. Functional cell biological experiments are ongoing to define the biological roles of these proteins. In particular, we have exciting evidence that PSB7 equips the tumor with an immune escape mechanism to evade MHC class I peptide presentation of tumor antigens, for the first time linking epithelial proteasome function to tumor immunology.

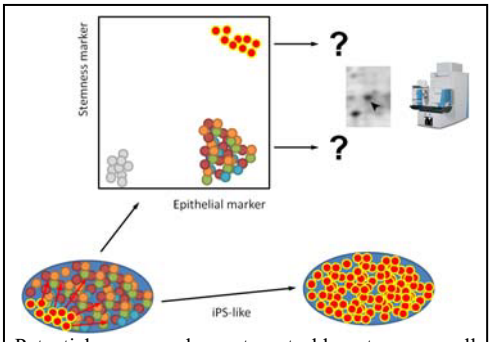


Preliminary experimental data showing immunohistochemical localization of the new candidate biomarker PSB7 expression in a colon adenocarcinoma. Presence of the specific protein is indicated by the amount of brown staining. Nuclei were counterstained with hematoxylin for visualization purposes. (A, B) Normal colonic mucosa with low levels of PSB7 expression. (C) **Markedly increased cytoplasmic and nuclear PSB7 expression in the patient's adenocarcinoma.** (D) Elevated PSB7 expression in carcinoma present in pericolonic lymphatic vessels; note the surrounding adipose tissue.

2. Cancer Stem Cells in Solid Tumors

We are particularly interested in isolating and studying the proteomes of tumor-promoting stem cells within solid tumors. Within a solid tumor, there may co-exist a variety of heterogeneous populations of neoplastic cells that, while genomically identical or highly related, differ widely in their epigenetic preoteomic make-up. Furthermore, one or several of these subpopulations may possess self-renewing and/or differentiation-blocked properties rendering them tumor-propagating ("stem"-like) relative to other populations which may not possess propagating capability. The characterization of molecules that define cancer cells as tumor-propagating cells will have significant impact on diagnosis, treatment strategy, and treatment response monitoring.

We have begun to isolate tumor-propagating cells from solid tumors by sorting for expressed surface protein markers. One candidate is the the pentaspan glycoprotein prominin-1 (CD133). We are investigating how the functional proteomic compositions of CD133 low/negative and CD133 high/positive tumor cells differ and how this may be related to their propagating capacities. There is recent evidence that some liver metastases of colorectal carcinomas display low levels or absence of CD133. By contrast, tumor-propagating cells in the primary colorectal neoplasm typically express CD133. Thus, the comparison of proteomic network alterations between these two types of lesions may provide direct clues about the functional context, interaction network, and downstream signaling partners of CD133. Through whole proteome comparison, we also hope to identify new protein markers that distinguish propagating from non-propagating phenotypes.



Potential approaches to tackle tumor cell heterogeneity. (*i*) Sorting an subpopulation analysis (top path). (*ii*) Engineering of homogeneous designer cancer stem cells (bottom path) using a currently hypothetical iPS-like reprogramming approach.

Harvard Medical - Gewurz

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nonical NF-kB activation by the prototypic canonical NF-kB pathway agonists Tumor Necrosis Factor-alpha and Interleukin-1-beta. Thus, LMP1 studies promise to uncover general mechanisms that govern NF-kB activation. I am currently characterizing screen hits with the most robust effects on LMP1/NF-kB, with a particular focus on potentially druggable enzymes. In parallel, I am performing comprehensive siRNA analysis of the LMP1/non-canonical NF-kB pathway. To determine whether these LMP1/NF-kB hits also function in the prototypic lymphocyte non-canonical NF-kB pathways, validated siRNAs will be retested for effects on NF-kB activation by two prototypic cytokine agonists.

Tumor genome characterization technologies are uncovering numerous genetic lesions in lymphoma cells. Differentiating biologically meaningful 'driver' mutations from the many functionally inconsequential 'bystander' alterations remains a key challenge. Integration of siRNA and tumor genetic alteration data offers a powerful approach to identify novel oncogenes and tumor suppressor genes. I am currently determining whether the strongest LMP1/NF-kB pathway screen hits are targeted by copy number and/or coding region alterations in primary lymphoma samples and established cancer cell lines. The convergence of multiple mutations on a given NF-kB pathway component would provide compelling genetic evidence for its role in lymphoid malignancy.

Finally, in conjunction with the Broad Institute, I am performing high throughput chemical screens to discover novel small-molecule inhibitors of canonical and non-canonical NF-kB pathways. Potent NF-kB inhibitors will then be tested for selective effects on the growth and survival of NF-kB dependent lymphoma cells.

Your Support is Vital to our Mission

The Karin Grunebaum Cancer Research Foundation and the support we are able to give to the dedicated cancer researchers is solely dependent on donations from you.

To further demonstrate our dedication, we keep our overhead funding at **1%** – meaning that **99%** of your contribution will go directly to research.

Your tax-deductible contribution will directly help fund the cancer research effort, since almost 99% of our income is spent on supporting the researchers. Our Officers and Trustees are all unpaid volunteers, and the Foundation has no paid employees.

You can make your contribution via check by mail. Send to:

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85 Sherman Street, #8
Cambridge, MA 02140

Online Support via PayPal at www.grunebaumfoundation.org

RECIPIENTS AT WORK

Dr. Roehrl – Human Proteome Organization 2010 Meeting, Sydney, Australia

Dr. Roehrl was awarded a Young Investigator Award from the International Forum of Proteomics at the 2010 HUPO Meeting in Sydney, Australia. He presented on his lab's work on tissue proteomics and protein biomarker discovery in colorectal and lung adenocarcinomas. The annual HUPO meeting is the world's largest meeting of scientists engaged in human proteomics and biological mass spectrometry.