KARIN GRUNEBAUM
cancer research foundation

BOARD OF TRUSTEES

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Dear Friends of the Karin Grunebaum Cancer Research Foundation:

A Golden Anniversary (50 years) is a universal time to celebrate, and the year 2008 is our Foundation's Golden Anniversary. We have chosen this auspicious occasion to pay tribute to the 80 Fellows whom the Foundation has sponsored since its inception in 1958. The Trustees decided that the most appropriate way to honor these dedicated cancer researchers is by creating an interactive website on which each of the Fellows can be individually recognized and their accomplishments relayed to the entire on-line community.

I am very pleased to announce that the website is now complete, and can be seen at: www.grunebaumfoundation.org. I hope you are as pleased with the results as I am. As you will see on the website, there is a separate page for each Fellow to insert his or her photo, contact information, medical specialty, location and publications. Each Fellow's site is individually password protected, and each Fellow has the opportunity to "opt in" or "opt out" of having their information publicly displayed. I strongly encourage each Fellow to put as much information as possible on their individual site so that we may remain in contact with you, as well as publicize your achievements worldwide.

You will note that each of the Foundation's currently sponsored Fellows is prominently featured on the home page, along with a brief synopsis of their research effort. These current Fellows also have the opportunity to self-edit and update the information about the status of their work on a constant basis, so that we can follow their progress towards the research project's goals.

Another section of the website ("Focus on Research") highlights those Fellows who have continued their work in medical research either through academic appointments or in commercial settings. We are thrilled to know that the Foundation "sowed the seeds" for their on-going interest in medical research.

If you have any ideas on how we can make the website even better, I welcome your suggestions. You can e-mail me at:

steven.wallach@grunebaumfoundation.org.

Of course, no charitable foundation web-site could be considered complete without a means of processing on-line donations, so we have also provided that capability. I hope each of you reading this newsletter will take advantage of this easy opportunity to help the Foundation celebrate its Golden Anniversary by making a significant contribution so that we can continue to fund cancer research efforts for at least another 50 years (or until this dreaded disease is finally vanquished). We have no other means of support, and we depend entirely on your donations to support our dedicated research Fellows.

I look forward to your comments and to your support in the coming months.

Steven Wallach, Chairperson

www.grunebaumfoundation.org

News from Harvard Medical School

Our sincere gratitude goes to our friend and fellow board member, Professor Jules Dienstag, who is stepping down from the Foundation Board due to an administrative change at Harvard Medical School. We thank him for his years of dedication and service to the foundation and will miss his presence and keen insights in helping the Foundation accomplish it's objectives.

As a result of the administrative change, Professor Thomas Michel has been named the Dean for Education and will be working across education levels coordinating among college, undergrad medical, graduate medical, PhD, MD-PhD, faculty and others. Please join the Board in welcoming our newest member.

Thomas Michel is the Dean for Education at Harvard Medical School, where he serves as Professor of Medicine and Biochemistry and Federman Chair in Medical Education, and is a Senior Physician in Cardiovascular Medicine at Brigham and Women's Hospital.

Michel was born and raised in Portland, Oregon, and received his undergraduate degree in Biochemical Sciences from Harvard College. He received his PhD in Biochemistry and his MD degree from Duke University, where he studied in the laboratory of Professor Robert Lefkowitz. He returned to Boston for his clinical training in medicine and cardiology at Brigham and Women's Hospital, and completed postdoctoral training in the laboratories of Professors Eva Neer and Jonathan Seidman at Harvard Medical School. He was then appointed to the faculty at Harvard Medical School, where he has worked as an active researcher, teacher and clinician for many years.

Author of more than 120 peer-reviewed research papers, Michel has led studies on the molecular mechanisms that control the endothelial nitric oxide synthase (eNOS), a key enzyme in cardiovascular homeostasis. Michel's laboratory was the first to clone and characterize eNOS more than a decade ago, and has been at the forefront of many major discoveries in this area ever since. Michel described the mechanisms for the control of nitric oxide signaling by eNOS post-translational modifications and by targeting to plasmalemmal caveolae. His laboratory found that eNOS is also expressed in cardiac myocytes and modulates myocyte function. Michel's elucidation of a caveolin-calmodulin regulatory cycle, in which eNOS-caveolin interactions are dynamically regulated in response to receptor activation, established a paradigm for the study of caveolin-regulated signaling pathways. Michel discovered that the platelet-derived lipid sphingosine 1-phosphate (S1P) is an important agonist for eNOS. Michel has also pioneered analyses of the dynamics of eNOS subcellular targeting using biochemical, biophysical, and cell imaging approaches, and his studies have revealed key regulatory interactions between plasmalemmal caveolae and the actin cytoskeleton. He and his laboratory discovered that eNOS undergoes reversible receptor-regulated S-nitrosylation, revealing a key role for S-nitrosylation in modulation of eNOS bioactivity. His lab has unraveled many of the complexities of eNOS regulation using novel molecular biological, biochemical, and cellular imaging approaches. In collaborative studies, he participated in a research team that discovered that firefly flashing is regulated by nitric oxide. His laboratory is currently funded by research grants from the National Institutes of Health, and Dr. Michel also serves as Principal Investigator of an NIH Program Project Grant studying the molecular mechanisms underlying diabetic vasculopathy.

Michel has garnered numerous prizes, including the John J. Abel Award in Pharmacology. He served as Chairman of the NIH Pharmacology Study Section, and has served on several editorial boards, including the Journal of Biological Chemistry and the Journal of Clinical Investigation. He has been elected to membership in the American Society of Clinical Investigation, the American Association of Physicians, and the Association of University Cardiologists, and is a Fellow of the American College of Cardiology. Michel is also a cardiologist and clinician-teacher. He served for 8 years as Chief of Cardiology at the VA Boston Healthcare System, where he led the most active VA Cardiology Section in the United States. He is currently a Senior Physician in Cardiovascular Medicine at Brigham and Women's Hospital, and was recently named to the Federman Chair in Medical Education at Harvard. He was the recipient of the 2005 Eugene Braunwald Teaching Award at Brigham and Women's Hospital, an award presented annually to the most outstanding teacher in clinical cardiology.

Michel has made many contributions to medical, graduate, and undergraduate education at Harvard University. In 2008, he was appointed the first Dean for Education at Harvard Medical School, in which role he is engaged in leadership of a broad range of educational programs at Harvard Medical School and its teaching hospitals, and across Harvard University and beyond.

His lab web site is: http://michel-lab.bwh.harvard.edu/index.html.

Harvard Medical School's relationship with the Karin Grunebaum Cancer Research Foundation began in 1966 when the Foundation funded its first Harvard fellow. By 1979, the Foundation had increased its funding to two Harvard Medical School fellows a year. Currently, the fellowships are open to junior faculty at HMS, though medical students were eligible in the past.

Harvard Medical School faculty members are eligible to become Karin Grunebaum Faculty Research Fellows during their first five years of faculty appointment in laboratory or translational cancer research. The HMS fellows to be funded by the Grunebaum Foundation are selected annually by a Harvard Medical School faculty committee in consultation with one or more Trustees of the Foundation, and each receives $40,000 for the first year. Applications are accepted for a second year extension.

The first Grunebaum Foundation fellow at Harvard Medical School was medical student Michael Droller, whose cancer-related research focused on detecting human bladder carcinoma cells in voided urine samples. When Dr. Droller graduated in 1968, he continued his work in urologic oncol-

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News from Boston University Medical School

For more than 30 years the Karin Grunebaum Cancer Research Foundation has generously supported the training of physician-scientists in cancer biology and research at Boston University School of Medicine (BUSM). "The continued commitment of the Karin Grunebaum Cancer Research Foundation in supporting fellows and junior faculty not only provides support for cancer research but also develops the next generation of faculty committed to preventing and treating cancer," explained Karen Antman, M.D., Provost of the Boston University Medical Campus and Dean BUSM. "We are deeply indebted to the Grunebaum Foundation. The Foundation's mission has been to support cancer research while honoring their mother's memory."

This partnership between the Foundation and BUSM has grown over decades. Today the relationship is multifaceted and includes:

Fellowships: From 1979 to 2006 38 fellowships were awarded to M.D./Ph.D. students. Competition for fellowships was intense with four times as many applicants applying as awardees. When the Foundation established the Grunebaum Chair and Professorship of Cancer Research, BUSM chose to continue the Fellowship Program with institutional support.

Chair and Professorship of Cancer Research: In 2002 the Foundation established the Karin Grunebaum Chair and Professorship in Cancer Research at BUSM with a $2 million endowment. This Chair was established to support a physician /scientist actively engaged in cancer research and in the education of medical and graduate students in cancer research. Douglas Faller, M.D., Ph.D., director of the Cancer Research Center at Boston University Medical Campus, was named as the first recipient of the professorship. Faller, who is also professor of medicine, pediatrics, biochemistry, microbiology, pathology and laboratory medicine at BUSM, has made numerous contributions to the understanding of how cancer emerges and spreads. He has published more than 300 peer-reviewed articles in medical and scientific literature.

Junior Faculty Focus: In 2006 the Foundation modified its focus and chose to assist junior faculty members already involved in clinical or translational cancer research rather than fund student researchers. This represented a major change in direction for the Foundation, and was based on the premise that medical students' areas of research interest may change or that they may choose to pursue careers in clinical practice. Faculty members have already committed themselves to a career in cancer research and therefore stand a greater chance of making an important contribution to cancer research, as well as increasing the impact of the Foundation's support.

In 2007 the first faculty award was given to M. Isabel Dominguez, Ph.D., assistant professor of Hematology/Oncology in the Department of Medicine. The current recipient is Julia Yaglom, Ph.D., assistant professor in the Department of Biochemistry. Dr. Yaglom's work focuses on how Hsp72 regulates DNA Damage Response (DDR) in tumor cells.

"The Grunebaum Foundation's support for cancer research at Boston University has been an extremely important part of our institutional cancer research program for decades. Generations of M.D. / Ph.D. candidates have gone on to have successful careers in cancer research as well as other medical fields. More recently, the Grunebaum Foundation endowed the first professorship for cancer research at BUSM, an endowed chair that will support the cancer research efforts of many distinguished scientists and their trainees over the years. The newest focus of the Foundation, providing support for young faculty just beginning their independent careers in cancer research is of particular significance due to the limited funding currently available for new cancer investigators, and for medical research in general," explained Faller. "We are all deeply indebted to the Foundation and the Grunebaum family."

News from Harvard Medical School *(continued from previous page)*

ogy, eventually becoming a professor of urology and oncology at Mount Sinai School of Medicine, as well as chairman of the Department of Urology, editor-in-chief of "Urologic Oncology: Seminars and Original Investigations," and editor of the text, "The Surgical Management of Urologic Disease: An Anatomic Approach," a standard in the field.

Cristina Ferrone, a Grunebaum Fellow and Harvard Medical School instructor in surgery at Massachusetts General Hospital, currently is focusing her research on the immune system's response to pancreatic cancer. Funding from the Grunebaum Foundation paved the way for her to participate in the American Society of Clinical Oncology's 2008 Gastrointestinal Cancers Symposium, where she reported on BRCA germline mutations in Ashkenazi patients with pancreatic adenocarcinoma.

"Harvard Medical school is deeply grateful to the Karen Grunebaum Cancer Research Foundation for its unwavering support of cancer research by medical students. Our first three awardees are now department chairs in three outstanding medical schools. The list of our fellows since then brings additional luster to our school and, we hope, to the Foundation," said Dr. Daniel Federman, the Carl W. Walter Distinguished Professor of Medicine at Harvard Medical School, and past chair of the Grunebaum Foundation board of trustees. "We treasure the connection."

Recipients of the Grunebaum Fellowship have been given the opportunity to explore the fields of basic, translational and/or clinical cancer research for a full year at pivotal points of their medical careers. Harvard Medical School appreciates the part the Karin Grunebaum Cancer Research Foundation plays in the development of junior faculty and their cancer-related research, and congratulates the Foundation on its 50th anniversary.

Judith Montminy
Associate Director of Public Affairs, Public Information
Harvard Medical School

From the Fellows

Boston University Medical School

Julia Yaglom, PhD, Research Assistant Professor
Department Biochemistry, Boston University Medical School

Activation of senescence program in tumor cells suppresses the major DNA repair factor, H2A.X.

Radiation and genotoxic drugs are the most common agents in treatment of various forms of cancer. However, despite the progress in radio- and chemotherapy, resistance of cancer cells to the treatment is the main obstacle in tumor control. Among various endogenous factors of tumor radio- and chemoresistance, heat shock proteins (Hsps) apparently play an important role. Accordingly, various tumors express higher levels of Hsps comparing to normal tissues, and expression of Hsp72 in breast and colon cancer correlates with invasiveness, resistance to drugs, and poor prognosis. However, the role of Hsps in tumorgenesis remains poorly understood.

Our previous data demonstrated that in several human tumor cell lines knockdown of Hsp72 or Hsp27 activated p53-dependent of p53-independent senescence. Here we demonstrate that this activation of senescence program leads to impairment of important pathway of DNA repair and associates with sensitivity to DNA damage. The cellular DNA damage response involves sensing of DNA damage by a class of PI3K protein kinases, ATM, ATR and DNA-PK, followed by activation of Chk1 and Chk2 kinases that cause temporal cell cycle arrest via p53/p21 and CDC25/cdk1 pathways. In parallel, phosphorylation of specific histone isoform, H2A.X, is activated to promote assembly of DNA repair complexes at the damaged sites at chromosomes.

We showed that downregulation of Hsp72 markedly sensitizes human carcinoma cells to gamma-radiation and doxorubicin, and this sensitization was associated with decreased formation of radiation-induced foci by histone gH2AX, indicating a defect in activation of the DNA damage response. Inhibition of H2AX phosphorylation and sensitization to DNA-damaging stresses was not a direct effect of Hsp72 downregulation but rather a hallmark of cells with activated senescence programs. Indeed, knockdown of Hsp27, direct activation of p52 with small chemical Nutlin-3, or expression of cell cycle inhibitors, p21 or p16, activated senescence, inhibited H2A.X phosphorylation and sensitized cells to DNA damage. Importantly, expression of Her2 or RAS oncogenes in normal human mammary epithelial cells, which also activated senescence program, blocked H2A.X phosphorylation and sensitized cells to DNA damage.

Further we showed that induction of senescence either through downregulation of Hsp72 in transformed cells or through expression of activated oncogenes in normal cells was accompanied with the reduced expression of histone H2A.X, while activation of DNA damage sensor kinases, ATM or ATR, was normal. To elucidate whether reduced expression of H2A.X can account for increased sensitivity of cells to DNA damaging stresses we have depleted H2A.X in human colon tumor cells HCT 116. We observed that even modest two-fold depletion of H2A.X in HCT 116 cells markedly sensitizes these cells to common chemotherapeutic drug doxorubicin. Interestingly, upon doxorubicin treatment induction of senescence promoting factor, p21 was significantly increased in H2A.X depleted cells as compared to parental HCT116 cells. Therefore, it appears that Hsp72 depletion triggers positive feed back loop, i.e. Hsp72 depletion lead to senescence and subsequent defect in DNA damage response due to reduction in the levels of the major double stranded DNA repair factor, H2A.X, which in turn stimulates senescence through induction of p21. This feed back loop may explain the importance of Hsp72 in promoting tumorgenesis. Presently we are planning to investigate whether similar feed back loop operates in vivo.

Massachusetts General Hospital

Dirk Bausch M.D., Research Fellow
Massachusetts General Hospital, Department of Surgery

Sonic hedgehog in tumor angiogenesis - a potential target for anti-angiogenic therapy in pancreatic cancer?

Despite all efforts in the last decades, pancreatic ductal adenocarcinoma (PDAC) still is a devastating disease: It is the 4th leading cause of death from neoplasia in the U.S. and other industrialized countries, with cure rates of less than 5% and an overall median survival of less than 1 year[1]. However, at the time of diagnosis, only about 20% of pancreatic tumors can be surgically resected. Adjuvant and palliative chemotherapy provides clinical improvement in only about 25% of patients. Therefore, new therapeutic strategies that notably lack cross resistance with established treatment regimens are much needed.

A marked host stroma response, termed 'desmoplasia', is a universal feature of PDAC. This tumor-stroma interaction is a specific requirement for the de novo formation of blood vessels, termed angiogenesis. Even though the tumor cells themselves secrete angiogenic factors, such as vascular endothelial growth factor (VEGF), the stroma is thought to play an equally important role in the induction and maintenance of an angiogenic phenotype.

Angiogensis itself is necessary for tumor growth beyond a microscopic size as well as metastasis formation[2-4]. The use of anti-angiogenic agents should consequently add another useful tool to the chemotherapeutic regimen. To date, however, clinical trials using anti-angiogenic agents have been of limited success[5]. Therefore, new anti-angiogenic strategies need to be pursued.

Overexpression of the morphogen Sonic hedgehog (Shh) is widespread in PDAC and required for its growth and maintenance[6,7]. One mechanism by which Shh supports tumor

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growth may be by promoting tumor angiogenesis: In non-tumor model systems, such as wound repair, the secretion of VEGF and other angiogenic factors from stromal fibroblasts is induced by Shh[8], which also directly stimulates endothelial cells and capillary morphogenesis[9,10]. Another mechanism by which Shh could affect tumor angiogenesis is through matrix metalloproteinases (MMP). MMPs are another important angiogenic factor involved in remodelling of the stroma and interacting with VEGF[11-13]. MMP expression in tumor cells and fibroblasts can be induced by Shh, but the contribution of this effect to angiogenesis has not yet been defined[14].

We assume that Shh plays an important role in angiogenesis in PDAC by *direct* effects on the endothelium, as well as *indirect*ly by modifying the tumor microenvironment (stroma) to secrete proangiogenic factors such as VEGF and MMPs. The inhibition of Shh should thus be a promising novel therapeutic approach in the treatment of PDAC by inhibiting tumor cell growth as well as tumor angiogenesis.

To test our hypothesis, we evaluated human PDAC samples and cell lines for their Shh expression and found a several hundred- to a thousand-fold overexpression together with the upregulation of downstream transcriptional activators and target genes. Furthermore, in a small scale study using mice bearing human PDAC xenograft tumors, treatment with Hh pathway inhibitors resulted not only in a significant decrease in tumor size but also in a decreased tumor vascularity.

Our current aims are to determine if Shh indirectly promotes tumor angiogenesis in PDAC by enhancing the expression of VEGF and MMP-9 in the tumor stroma in vitro using co-culture systems and in vitro angiogenesis assays. To confirm these in vivo, we plan to use mice bearing human PDAC xenograft tumors together with Shh, VEGF and MMP inhibitors. Furthermore, we plan to evaluate the direct effects of Shh on endothelial cells during tumor angiogenesis in PDAC in vitro and to confirm these findings in vivo using the aforementioned methodology. The answer to these questions will not only allow us determine the role of Shh in tumor angiogenesis in the context of PDAC, but also help to determine the feasibility of a potential combined anti-angiogenic therapeutic approach targeting more than one angiogenic pathway.

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Cristina R. Ferrone M.D., Instructor in Surgery
Harvard Medical School

The American Cancer Society predicts 37,680 new cases and 34,290 deaths from pancreatic adenocarcinoma in 2008[1]. Less than 15% of patients diagnosed with pancreatic adenocarcinoma will undergo surgical resection. Despite extensive research, adjuvant chemotherapy provides a marginal benefit and surgical resection remains the only potential cure. Immunotherapy with surgical resection could provide a novel approach to the treatment of pancreatic adenocarcinoma.

Our first aim was to evaluate the prognostic significance of tumor-infiltrating immune cells in patients with pancreatic adenocarcinoma in the setting of known clinicopathologic factors and recurrence/survival data. Specifically, how the presence and type of immune infiltrates modifies tumor stroma/tumor cells and affects the metastatic potential of pancreatic cancer cells. The tissue array for this component has been constructed and the slides are currently being stained. Preliminary data demonstrates an increase in the regulatory T cell infiltrate. Regulatory T cells are inhibitory T cells which decrease the strength of the immune response to the tumor.

Our second objective was to evaluate the role of soluble major histocompatibility complex class I related gene A (MICA) as well as antibodies to MICA in the peripheral circulation of patients with pancreatic adenocarcinoma. No significant levels of soluble MICA were demonstrated in the peripheral circulation of patients with pancreatic adenocarcinoma. However, antibodies to MICA are present. Interestingly, with higher stages of disease there are lower levels of circulating antibodies. This may indicate that with a higher tumor burden the immune system is sensitized to the tumor antigens and no longer recognizes it as foreign. This hypothesis is undergoing further investigation.

Third (New)Objective

Current cancer immunotherapy protocols provide only a small percentage of patients an objective benefit. The limited benefit may be due to the tumor's ability to escape immune surveillance and induce immune tolerance. Multiple mechanisms by which the tumor escapes the immune system have been proposed, including the suppression of tumor specific T cells by regulatory T cells (Tregs). Tregs are suppressive CD4+Tcells, which express high levels of CD25 (IL-2alpha receptor subunit), CTLA-4 and the glucocorticoid-induced tumor necrosis factor alpha-receptor on its surface. Up regulation of CTLA4 on the surface of Tregs through cell-to-cell contact can suppress the activation and expansion of effector cells to both normal self and tumor antigens.

Liyanage et al[2] demonstrated a significant increase in the circulating T reg population (CD4CD25) as a percent of the total CD4 population in patients with pancreatic adenocarcinoma (8.2% vs. 13.2%, p=0.015). Also, the ratio of CD8+T cells to Tregs was low. Hiraoka et al[3] histopathologically evaluated 198 pancreatic ductal adenocarcinomas, 51 intraductal papillary mucinous neoplasms and 15 non-neoplastic pancreatic lesions. A higher density of Tregs in the primary tumor was associated with an advanced tumor stage (p=0.029) and a higher tumor grade (p<0.001). Patients with a lower density of Tregs in their tumor also had a better overall survival (p<0.001) with a mean survival of 13.4mo vs. 26.2mo. The infiltration of Tregs continued to be a prognostic predictor in the setting of known clinical factors such as tumor grade and margins (TNM). Our preliminary results using flow cytometry of peripheral blood in patients with resectable pancreatic adenocarcinoma are demonstrating similar results. Therefore, it is thought that Tregs play a critical role in maintaining self-tolerance, but also facilitate a level of nonresponsiveness which allows the tumor to grow.

We hypothesize, based on published data and our own preliminary data, is that patients with pancreatic adenocarcinoma have a larger Treg population than patients without cancer. Our third objective is to further evaluate the contribution of Tregs in patients with resectable pancreatic adenocarcinoma utilizing immunohistochemistry and flow cytometry.

We have submitted a request to Pfizer for anti-CTLA-4 antibody. We are proposing a clinical trial utilizing an anti-CTLA-4 antibody in combination with radiation therapy. Preclinical studies demonstrate that administration of an anti-CTLA-4 antibody results in an expansion of effector CD8+ Tcells, which could potentiate the immune response. By administering an anti-CTLA-4 antibody we could increase the ratio of CD8 + T cells to Treg cells, which has been shown to be an important predictor of the immune response in other tumors. We hypothesize that neoadjuvant radiation would result in cell death of the primary pancreatic adenocarcinoma. This would act as an autologous tumor vaccine. Administration of an anti-CTLA-4 antibody would strengthen the patient's immune response to the primary adenocarcinoma by altering the effector to regulatory T cell ratio, and help generate a stronger immune response to the pancreatic adenocarcinoma.

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