Karin Grunebaum Cancer Research Foundation

Founded in 1958

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Karin Grunebaum Cancer Research Foundation
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Dear Friends of the Karin Grunebaum Cancer Research Foundation:

As I write this note, it's been almost one year since I assumed the leadership of the Foundation, and it's been a most interesting time. First, for the first time since 1992, our elected officers are all members of Karin Grunebaum's family. I hold the Chairperson position as Karin's oldest son; my sister, Carol Kelly, is the Treasurer; and my daughter (Karin's grand-daughter), Shelby Schultz, is the Secretary. Additionally, my other sister, Yvonne Grunebaum, has been responsible for publishing the Foundation's newsletter.

Our non-familial Trustees continue to represent the highest levels of medical research and medical education in the world, and I am thrilled to work with them and appreciate all their efforts on behalf of the Foundation to insure that we provide the Fellows with a top quality experience.

Those of you who remember last year's newsletter will recall the three initial objectives I laid out for the Foundation:

1. Increasing the funding for the Fellowships so that additional research opportunities are made available;
2. Making the Karin Grunebaum Fellowship program an on-going personal and professional experience for the current and former Fellows by fostering social and professional interaction between them; and
3. Insuring that the cancer research projects undertaken by the Karin Grunebaum Fellows are brought to a final conclusion, unhampered by individual researcher's time constraints.

With regard to the first listed objective, I am pleased to report that the Foundation recovered over $240,000 of assets, which were intended for the Foundation, but were on deposit at the Harvard Medical School. We are currently trying to recover a similar amount of money which was intended to inure to the Foundation, but is on deposit at Massachusetts General Hospital.

We have also changed the Foundation's financial advisor to Fidelity Investments, which has resulted in an immediate annual savings of investment expenses of over $12,000. These savings, coupled with the money recovered from Harvard, have allowed us to fund an additional researcher, thereby achieving the first objective.

With regard to the second objective, numerous current and past Karin Grunebaum Fellows from Boston University and Harvard enjoyed a reception the Trustees held in their honor in Cambridge last October. In May, all current Karin Grunebaum Fellows met at Harvard to present a synopsis of their projects to the Trustees and to each other. They also heard a superb presentation by Professor Douglas Faller, who holds the Karin Grunebaum Chair in Cancer Research at Boston University. I look forward to more of these interactive get-togethers, and think the dynamics of such gatherings can only help us achieve the goal of shared knowledge in the fight against cancer.

With regard to the third objective, I plan to ask the Trustees at the next Foundation meeting in October for suggestions on how to insure that research projects funded by the Foundation are taken to completion regardless of the researcher's own time constraints.

As always, I welcome your input.

Finally, as part of our on-going effort to raise funds, I again ask each of you (especially former Fellows) to please make a donation to this very worthy charitable organization, which uses its resources to "invest in people."

Steven Wallach, Chairperson
Karin Grunebaum Cancer Research Foundation

FROM THE TRUSTEES

The trustees of the Foundation want to remind everyone that the support given to the students is possible only through private donations and the generous contributions from those who have benefited from the foundation and those who are dedicated to the hope for a cancer-free world. We ask that you send your tax- free donations to: KGCRF 85 Sherman Street #8, Cambridge, MA 02140. Tax ID # given upon request.

Environmental Toxins and Breast Cancer

Nicolas Currier, M.D. / Ph.D. candidate
Grunebaum Fellow 2005
Boston University School of Medicine

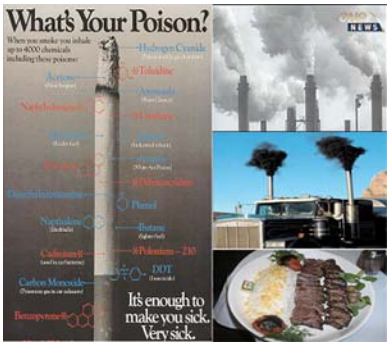
Breast cancer represents the second leading cause of cancer deaths in women, and while research has made significant gains in our understanding of familial breast cancer syndromes and inheritance genes (such as BRCA1 and BRCA2), these account for only ~5% of breast cancer cases. The majority of breast cancers are spontaneous, however, we still have little understanding of the mechanisms behind spontaneous mammary tumorigenesis. Epidemiologic data have shown significant geographic variation in the incidence of breast cancer, which suggests that environmental and dietary factors play a significant role, particularly carcinogens such as polycyclic aromatic hydrocarbons (PAH). Additionally, PAH have been implicated in mammary tumorigenesis by epidemiological and laboratory studies. DMBA (7,12-dimethylbenz[a]anthracene) is a prototypical PAH that has been used to promote tumors in laboratory animals. Thus, my research has focused on using a model of sporadic breast cancer based on exposure to DMBA to study biochemical mechanisms that may be at the root of spontaneous, non-familial, breast cancers.

Using oral doses of DMBA we were able to generate mammary tumors in 75% (15 of 20) of female mice used in the study. In collaboration with the Sherr, Sonnenshein and Xiao labs at Boston University School of Medicine, we demonstrated up-regulation of many important proteins and signaling pathways with established roles in breast cancer, such as the aryl hydrocarbon receptor, Pin1 protein, and NF-kB components. Most relevant to our work in the Seldin lab, however, we demonstrated significant up-regulation of b-catenin and CK2 proteins, cyclin D1 mRNA, and other components of the Wnt pathway, a biochemical signaling pathway with a well-established role in both embryonic development and certain cancers, such as colon cancer. More recently evidence has emerged linking Wnt signaling with breast cancer. I have written up these findings, and they have been accepted for publication in the journal *Toxicologic Pathology*.

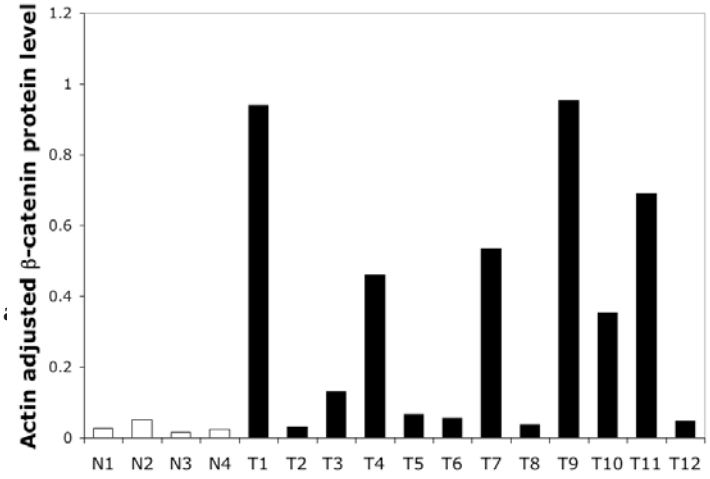
In order to continue to try to establish the role of Wnt signaling in carcinogen-induced mammary tumorigenesis, I have now begun two new experiments designed to confirm the presence of Wnt signaling in DMBA induced mammary tumors, and to investigate the possibility that these carcinogen-transformed cells depend upon CK2 and Wnt signaling to survive. The first experiment involves the use of transgenic mice that express a green fluorescent protein (GFP) in cells that have active Wnt signaling. We have already established that these mice properly express GFP in the appropriate tissue (those known to have active Wnt signaling) and we will soon be dosing these mice with DMBA, and evaluating the subsequent tumors for GFP expression. My second experiment involves growing DMBA-transformed mammary epithelial cells in culture. I will then attempt to inhibit the growth of these transformed cells with specific CK2 and Wnt signaling inhibitors.

Establishing a link between environmental carcinogens and specific biochemical signaling pathways may allow us to begin to understand the mechanisms behind spontaneous breast cancers and this could eventually lead to new therapies.

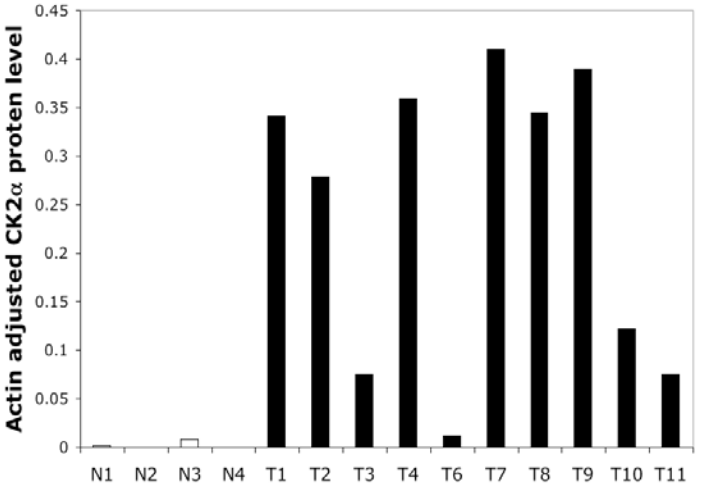
Environmental Sources of Polycyclic Aromatic Hydrocarbons



Up-regulation of b-catenin in DMBA induced mammary tumors (black bars) as compared to normal mammary glands (white bars)



Up-regulation of CK2 in DMBA induced mammary tumors (black bars) as compared to normal mammary glands (white bars)



Needs title for article and

Tim Daskevich
Grunebaum Fellow 2005
Harvard Medical School

Since its introduction ten years ago, Prostate Specific Antigen doubling time (PSA-DT) has been established as one of the most powerful prognostic and predictive indicators in the prostate cancer literature. PSA-DT is based on a simple principle: that rate of growth of Prostate Specific Antigen (PSA), the blood marker that reflects amount of prostate tissue in the body, should be an estimate of the aggressiveness of the cancer. The "doubling time" is one such measure of the rate of PSA growth, which specifically looks at how long it takes for PSA to double in number. In theory, a short PSA-DT (i.e. doubling of PSA over a short time period) should reflect more aggressive disease. This hypothesis has been proven time and again in many prostate cancer disease states: short PSA-DT has been associated with quicker time to disease progression or higher rates of prostate cancer-specific mortality when measured before initial treatment (i.e. at the time of diagnosis), at the time of recurrence after treatment, and at androgen independence (i.e. a later stage of advanced disease).

In February 2005, in conjunction with Dr. William Oh at the Lank Center for Genitourinary Oncology at Dana Farber Cancer Institute, I designed a retrospective study that assesses the ability of PSA-DT to predict success of taxane chemotherapy in patients with advanced prostate cancer. The study involves 221 prostate cancer patients treated with taxane chemotherapy at the Dana Farber, and it specifically looks at PSA-DT at both the time of androgen independence and directly before chemotherapy. This study comes at a particularly important juncture in the history of chemotherapeutic treatment for advanced prostate cancer, as taxane chemotherapies were recently shown to be the first chemotherapeutics to extend life in prostate cancer patients. Although they have since become the standard of care, there are no known indicators that can predict success of these treatments, which have success rates of around 50%. If PSA-DT proves to be a strong predictive indicator of success of taxane chemotherapy, it will identify patients who are likely to benefit from these treatments and target others for early entry into clinical trials, which will offer them better chances of success. It will also provide both patients and doctors with more information when considering the risks and benefits of initiating this type of chemotherapy, which is associated with considerable side effects. Currently, this study is in the data analysis phase, and we hope to have final results by November 2005.

In addition to this study, I have also used my time at the Dana Farber to work on many other exciting projects. I am currently finalizing revisions on a manuscript that explores the problems with rough estimation of PSA-DT in the clinic and the importance of mathematical method in such calculations. I also am working with Dr. Oh on a review article about hormonal therapies in advanced prostate cancer, which will be published in Current Opinion in Urology in May 2006. I have also written a case report that raises questions about the mechanism of drug failure in prostate cancer patients treated with Gonadotropin-Releasing Hormone Agonist Depots; this report has been submitted for publication and is currently under review. Lastly, since March, I have been working with a computer programmer to program PSA-DT calculations into Dana Farber's CRIS prostate cancer database, so that when a doctor sees a prostate cancer patient in clinic, the relevant PSA doubling times are automatically calculated. This will

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Microscopy: An Art of Science

Grace F. Monis, M.D. / Ph.D. candidate
Grunebaum Fellow 2005
Boston University School of Medicine

I was first drawn to the use of microscopes for research during the first two years of medical school. During histology and microbiology, I was amazed by the vast microscopic world around and within us. As a scientist, I was attracted to the "facts" of science, such as - cells in the walls of capillaries are simple squamous in shape or gastrointestinal bacteria that cause food poisoning are more likely to be a Gram stain negative species. As an artist, I was lured by the beauty. Images of cells can be appreciated as one would appreciate architecture – well designed, elegant, strong and efficient. Or as one would appreciate abstract art – brilliant colors, well framed and intricately composed.

When the chance to use confocal microscopy to study cellular interactions with a Multiple myeloma associated protein called AL-amyloid occurred during graduate school, I jumped at the opportunity. Cancer cells may produce and release unwanted products, such as proteins or metabolites that can spread to other organs of the body and disrupt their physiology. Organs commonly affected by AL-amyloid are the heart, lungs, kidney, GI and peripheral nervous system. Our lab labels amyloid protein with a fluorescent molecule which allows us to follow its interaction with live cells. Our experiments have shown that amyloid proteins are internalized and concentrated within cardiac fibroblasts in cell culture[1]. Internalized amyloid proteins are localized with membrane bound vesicles that traffic within the cell. These observations open up the possibility that damage done to cellular function may occur within the cell and in the extracellular environment. They also open up the possibility that cells may play an active part in the alteration of amyloid protein leading to its degradation or further toxicity. We are evaluating drugs to understand how they alter the disease process.

The Grunebaum Fellowship has been instrumental in furthering my use of the microscope to study AL-Amyloidosis. This past year of research has been a valuable experience toward becoming a physician scientist . Thank you for your support.

References

1. Trinkaus-Randall V, Walsh MT, Steeves S, Monis G, Connors LH, Skinner M: *Cellular Response of Cardiac Fibroblasts to Amyloidogenic Light Chains.* American Journal of Pathology 2005, 166:197-208

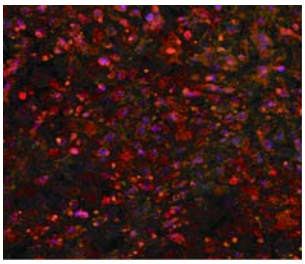


Figure 1. *Confocal microscope image (100X). Amyloid protein (light green pixels) is internalized by cardiac fibroblasts in culture. Nuclei (blue), Cytoplasm (red)*

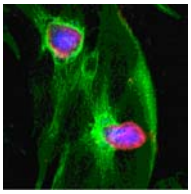
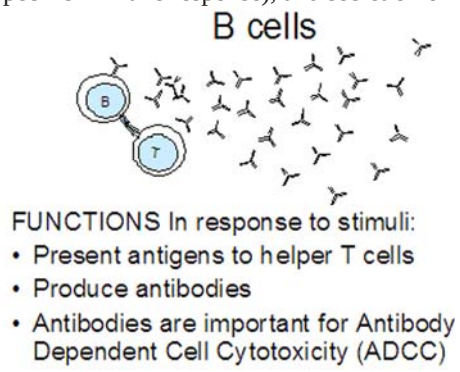


Figure 2. *Confocal microscope image (630X). Amyloid protein (magenta) is found to localize around the cell nucleus (blue). Actin cytoskeleton (green)*

Pretreatment of Splenic B lymphocytes with bacterial products induces and alternate signaling pathway

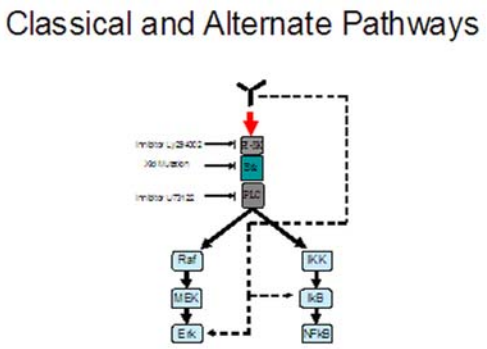
John R. Dye, M.D. / Ph.D. candidate
Grunebaum Fellow 2005
Boston University School of Medicine

B cells perform many important functions in the immune system. Along with T lymphocytes they make up the lymphocytic compartment of the immune system this is also commonly referred to as the adaptive immune system. Cells from this compartment are capable of memory with regard to specific antigens and in the process of an ongoing immune response their antigen specific receptors will "evolve," by utilizing a process known as receptor editing. Functions of the B cells specifically include: secretion of cytokines (small soluble molecules that are important for communication between cells), interactions with T lymphocytes including antigen presentation (this is important for triggering a specific immune response), and secretion of antibodies. B cells are also thought to interact with Bacteria and bacterial products (such as Lipopolysaccharide or LPS) during an ongoing immune response, by utilizing pattern recognition receptors on their cell surface. Most of these receptors belong to a family known as the toll-like receptor (TLRs) family. In addition B cells express a unique B cell receptor (BCR), which can bind its cognate antigen and induce a signaling cascade to activate the cell.



B cell signaling through the BCR critically depends on activation of the Nuclear Factor ˜B (NF˜B) family of transcription factors. Upon crosslinking of the B cell receptor a molecular cascade is initiated involving numerous kinases, lipases, and adaptor proteins. This cascade ultimately leads to the phosphorylation, ubiquitination and degradation of the Inhibitor of NF˜B (I˜B) which releases NF˜B and allows for its translocation into the nucleus. A number of molecules involved in the cascade have been linked into a conceptual framework known as the signalosome. The signalosome molecules have been shown to be indispensable for signaling and include; Btk, BLNK, PI-3K, PLC˜, and PKC˜. In addition B cells seem to depend on the signalosome for the majority of the MAP kinase signaling. Therefore if one disrupts any of these molecules by knockout, mutation, or chemical inhibition; signaling through the BCR is also disrupted such that one no longer observes NF-˜B activation or MAP Kinase activation. While investigating the role of the signalosome in Fas resistance, we inadvertently discovered an alternate BCR signaling pathway. Pretreatment of B cells with soluble CD40L allowed for signaling to bypass the signalosome. This was demonstrated in both Xid mice (which have a mutation in Btk) and in Ly294002 treated B cells (Ly294002 specifically inhibits PI-3K).

While the CD40L phenomenon was interesting, it may or may not be physiologically relevant. It is thought that in an ongoing inflammatory response to bacteria, viruses or even cancer; B cells may well be first responders. Therefore it is logical to presume that a B cell may encounter bacterial products such as: Lipopolysaccharide (LPS) and stimulatory unmethylated CpG DNA, prior encountering their cognate antigen, however some feel it is unlikely that B cells would encounter CD40L prior to activation through the BCR. I hypothesized that activation of B cells via innate stimuli might result in reprogramming of the BCR signaling cascade, due to similarities in downstream events between Toll-Like Receptors (TLRs) and CD40. B cells were treated with LPS and CpG in vitro for 24 hours. Stimulation in this manner created a bypass such that when the cells were treated with the PI-3K inhibitor Ly294002 or the PLC inhibitor U73122 they were able to maintain the normal signaling pattern in which both the NF-˜B and MAP kinase pathways were intact. I˜B- degradation was observed by western blot as well as IKK-˜/ phosphorylation, which occurs immediately upstream of I˜B- degradation. In addition NF-˜B was determined to have been induced to translocate to the nucleus in the bacterial product treated cells by EMSA. The MAP Kinase pathway was also found to be restored in the alternate pathway as determined by western blot for Erk-1/2 phosphorylation. To further elucidate the extent of the bypass events, Xid mice were used. It was determined that pretreatment with the bacterial products LPS and CpG were able to create the bypass here as well.



The impact of this work on understanding how a B cell may respond to bacterial infection is enormous. In addition by understanding how antigenic B cell responses can be altered (enhanced) by pretreatment with innate stimuli we may one day be able to enhance B cell responses to a variety of antigens and thereby modulate the ability of the immune system to eradicate diseases, both exogenous (such as bacterial and viral infection) and endogenous (such as cancer). In fact researchers have already begun to utilize this to some effect. As was observed with Coley's toxin at the turn of the last century, Bacterial products may be able to enhance immunological responses to cancer. Recently it has been demonstrated that treatment of patients with stimulatory unmethylated CpG in addition to an tumor specific antigen may have some efficacy (Curr Opin Mol Ther. 2001 Feb;3(1):15-24.). I hope to help shed light on the mechanisms by which these phenomena occur and perhaps by increasing our understanding of the mechanism we may be able to further enhance efficacy of immunologically based cancer therapy.

Daskevich *(continued from previous page)*

provide an important resource for prognostic and predictive information for every patient seen in clinic. We have achieved great success with this project so far, and we expect to implement it on a trial basis in the coming months.

I am very grateful to the Karin Grunebaum Research Foundation for giving me the opportunity to work at the Dana Farber over the past year. I have quite enjoyed working with the world-class researchers and clinicians at the Dana Farber, and I have come away from this experience more excited than ever about pursuing a career in genitourinary oncology surgery. I hope and expect that the work I've done this year will in some way benefit patients afflicted with prostate cancer, and I look forward to being a caregiver for these patients in the coming years.