KARIN GRUNEBAUM
cancer research foundation

December, 2011 • Volume 8

Founded in 1958

From the Chair

Dear Friends of the Karin Grunebaum Cancer Research Foundation:

An old adage is that if you are not moving forward, you are moving backwards. We always want the Foundation to move forward, as we have done for over 50 years. In that vein, we continually search for new ways to support cancer research.

As I wrote in last year's letter:

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.

In following up on this goal, during the past year the Foundation expanded its "investment in people" mandate by donating funds to support two entirely new programs. These sponsorships are in addition to our continuing support of junior faculty members engaged in cancer research at both Harvard Medical School and Boston University School of Medicine.

At Harvard Medical School the Trustees decided to support the Cancer Biology Area of Concentration Biological and Biomedical Services Ph.D. Program by donating funds to be used for the Fall 2011 Welcome Event, the Dana Farber Cancer Institute Lunch Speaker Series and the Student Data Club.

Boston University School of Medicine received funding from the Foundation for two undergraduate students doing summer laboratory research in cancer research since many of the undergraduate students who apply come from schools that do not have adequate laboratory resources for cancer research. The awards include required lab fees. The Trustees expressed hope that this experience would encourage the student to attend graduate school or medical school with an emphasis on cancer research.

While we continue to expand our methods of funding cancer research, unfortunately our income from donations has declined to the point where we have had to reclassify the Foundation as a *"private foundation"* instead of the *"public charity"* status we have held since the founding of the Foundation in 1958. **We are still a totally tax-exempt 501 (c) (3) charity, and all donations continue to be tax exempt**. The main effect resulting from this change is in the IRS reporting requirements and the amount of money which must be spent on the Foundation's mission.

I hope that we will soon be able to regain the "public charity" status, but that is solely dependent on the amount of donations received. We sincerely appreciate the donations received during the past year, and we ask each of you to please contribute further to our Foundation so that we can quickly regain our prior status. Please make your tax deductible contributions by mail or on-line.

Thank you.
Steven Wallach
Chairperson

www.grunebaumfoundation.org

From the Fellows

Harvard Medical School

Adam Bass, M.D., Instructor in Medicine

Over the past 30-40 years the incidence of esophageal adenocarcinoma (EAC) has risen dramatically, increasing 300-500% in the United States since 1970. During this time, esophageal cancer has shifted from being a disease dominated by alcohol and tobacco-associated squamous cell tumors located in the upper esophagus to a new entity, adenocarcinoma of the lower esophagus, a cancer preceded by gastric reflux disease. A parallel shift has characterized gastric cancer. Although total rates of gastric cancer have fallen in the United States during the 20th century, recent years have seen a marked increase in the incidence of gastric adenocarcinomas at the gastric-esophageal junction. Thus, this new phenomenon of gastric-esophageal junction adenocarcinoma has become an increasing public health concern, exacerbated by the poor survival associated with these cancers. Patients with esophageal and gastric adenocarcinomas have five-year survival rates of < 20%. However, the scope of this problem has not been met by an appropriately commensurate research effort. Significantly, our relatively poor understanding of the genomic alterations that drive these tumors is a roadblock to biologic understanding and therapeutic progress.

As a physician-scientist with a new independent laboratory at the Dana-Farber Cancer Institute, a primary goal of my new research team is to use the cancer genome as a starting point to better understand and, eventually, treat these diseases. We will harness our new ability to characterize cancer genomes by studying these discoveries in the laboratory to define the genes whose alterations underlie gastric and esophageal cancers. With this knowledge, we will be able to identify new therapeutic vulnerabilities in these tumors and gain key new insights into tumor biology. As I start my laboratory, I am fortunate to take part in leading large collaborative projects aimed at characterizing the genomic alterations in gastric and esophageal cancer. These studies are exciting and powerful, but they are merely a foundation for progress as they will require substantial laboratory follow-up to validate and understand the biologic functions of potential targets. Our ability to build off of these genomic discoveries is hampered greatly by the relative lack of model systems and resources for studying these cancers compared to those available for many other tumor types. Development of these essential models and systems is typically difficult to support from traditional granting agencies, yet this very work will be invaluable to our ability to translate the findings from genomic discovery. With support from the Grunenbaum Foundation, I hope to develop and apply in my laboratory new functional systems so we can identify and study those genomic events that are essential to esophageal and gastric adenocarcinoma.

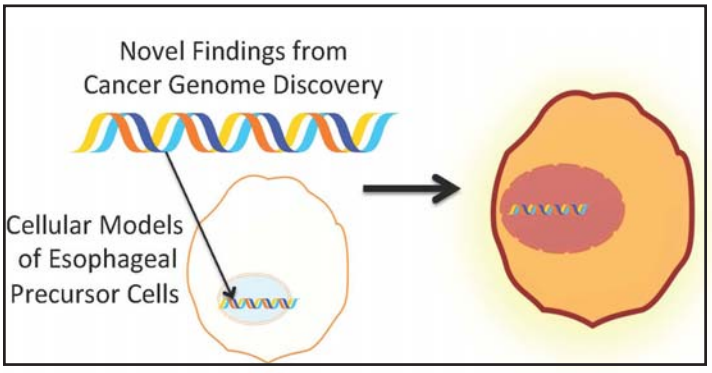
As an adjunct to the functional laboratory component of my new research group, we are heavily engaged in genomic characterization of esophageal and gastric adenocarcinomas.

I am co-leading the multi-institutional The Cancer Genome Atlas (TCGA) projects in gastric and esophageal cancers, projects aiming to generate the comprehensive analyses of the genomic features of these disease, studying both the genomic amplifications and deletions that develop by studying the DNA from these tumors using high-density single nucleotide polymorphism (SNP) microarrays. Further, we are pursuing the initial sequencing of all coding genes in these tumors.

However, the breadth of these data is immense and underscores the major limitation in our ability to translate the knowledge generated from modern genomics by first demonstrating which events are clinically relevant. The explosion in our ability to study the cancer genome far exceeds the capacity with which new putative oncogenes can be functionally interrogated. Thus, we risk losing valuable years before discoveries that have great potential to impact the treatment of patients can move forward. To confront this challenge, my laboratory will build a functional genomic effort in esophageal and gastric cancer so that we can best exploit our growing knowledge of cancer genomes. This work will require development and optimization of cellular and animal systems for functional testing, a general approach that worked effectively during my post-doctoral training.

Developing systems for these disease pose unique challenges. Modeling and evaluation of gastric and esophageal adenocarcinoma has been hampered in the past by the unique developmental origin of these cancers. These diseases both develop not out of normal esophageal or gastric tissue, but instead from tissue that has undergone intestinal metaplasia, a switch from their native histology to an intestinalized epithelium. The need to induce metaplasia as a pre-requisite to transformation has hampered the development of transgenic animal models for adenocarcinomas at the gastric-esophageal junction. Compounding this situation is the relative lack of established cancer cell lines derived

(continued on next page)



HARVARD *(continued from previous page)*

from adenocarcinomas the gastric-esophageal junction. Although many gastric carcinoma cell lines have been developed, these are predominantly generated from patients in Asia, where cancers generally differ from the gastric-esophageal junction cancers prevalent in our patient population.

Therefore, in these first years of my new laboratory, it will be essential to develop systematic approaches to studying novel genomic events in esophageal/gastric cellular models derived from intestinal metaplasia of the gastric-esophageal junction. These models will both allow functional genomic approaches to validate the candidate genetic lesions emerging from genomic discovery and can also then serve as model systems to pursue further functional characterization of validated genetic drivers of these diseases. The type of work that is being described here, the development of essential model systems and experimental methodology, is crucial to my effort of building a program in the study of these diseases and compete for further funding. I have great confidence that with this support, my new research team will be able to develop and use these model systems and approaches to help us take maximal advantage of the opportunity we have in the coming years to build off of genomic characterization to make substantial inroads into the understanding and treatment of these deadly diseases.

Boston University School of Medicine

Xaralabos "Bob" Varelas, Ph.D.
Assistant Professor, Department of Biochemistry, Boston University School of Medicine

Defining Polarity Cues that Promote Tumor Suppression

The asymmetry generated within cells, known as polarity, has a profound impact on the ability of cells to divide, establish cell fate and organize themselves into tissues and organs. Regulation of polarity is critical for developmental integrity, and altered polarity often results in severe malignancies, including birth abnormalities, or more commonly cancer. The most common types of cancers (nine out of ten cases of diagnosed cancers) arise from the deregulation of cells originating from epithelial tissues (referred to as carcinomas). Epithelial cells are polarized cells that are defined by an asymmetry known as apical-basal polarity, and the loss of this form of polarity is characteristic of carcinomas. Loss of epithelial polarity leads to the acquisition of properties that resemble mesenchymal cells, including the gain of migratory cellular abilities. Thus, this process is referred to as an epithelial-mesenchymal transition (EMT). Interestingly, EMT has been linked to the gain of tumor-initiating properties and to the transformation of cancer to a metastatic state, suggesting that cell polarity may influence these events. Despite a central role for cell polarity in human development and disease, the mechanisms by which cell polarity impinges on intracellular signals remain obscure.

Our recent work has revealed that proteins modulating polarity (polarity proteins) regulate the activity of a vital developmental signaling pathway known as the Hippo pathway. The Hippo pathway has an important role in establishing correct organ size, and does so primarily by controlling the localization and activity of two paralogous transcriptional regulators TAZ and YAP. Together TAZ and YAP play key functions in regulating cell proliferation, apoptosis, and stem cell properties. The importance of TAZ and YAP is highlighted by their ability to direct the first cell fate decisions in mammalian development, and that deregulation of nuclear TAZ/YAP activity strongly drives tumor initiation. Our data suggest that polarity proteins are eliciting growth control signals by influencing Hippo pathway and ultimately TAZ and YAP activity. We therefore hypothesize that the Hippo pathway serves as the conduit by which signals from polarity proteins suppress tumorigenesis, and thus deregulated associations between polarity and Hippo pathway cues are a contributing factor to cancer. Our group's goals are to define how cell polarity directs Hippo pathway activity, and to delineate how the uncoupling of these signals contributes to cancer. To achieve these goals we are undertaking two primary lines of study:

1) Define the molecular mechanisms linking polarity proteins to the Hippo pathway.

Distinct polarity protein complexes define apical-basal polarity, and our recent work has uncovered connections between these proteins and core Hippo pathway components. Using proteomic, biochemical and cell biology approaches we have set out to understand the molecular details linking polarity proteins to Hippo pathway regulation. Uncovering this relationship will provide much needed insight into how cell polarity signaling is linked to tumor suppression.

2) Characterize how deregulated Hippo pathway activity drives tumor initiation.

Our work indicates that nuclear TAZ and YAP activity has an important role in driving EMT, and thus we are examining how deregulation of TAZ and YAP contribute to this process. We are examining the transcriptional events regulated by these proteins and are assessing how cell polarity proteins impinge on TAZ and YAP activity. Such knowledge is critical for understanding how cancers arise, and thus our research will define novel tumor-initiating events that may lead to the identification of early cancer detection markers and more importantly contribute to the development of novel disease therapies.

Together our studies will provide key insight in the mechanisms directing epithelial organization and will provide a springboard for our long-term goal of defining tumor suppressor signals.

Boston University School of Medicine

Michael H.A. Roehrl, M.D.
Assistant Professor of Pathology and Laboratory Medicine, Boston University School of Medicine

Summary

Over the past year, my laboratory has worked on two main areas:
1. Cancer stem cells in solid tumors.
2. Metabolomics of colorectal cancer by ^{1}H and ^{1}H-^{13}C NMR (nuclear magnetic resonance) spectroscopy.

Over the past year, funding from the Grunebaum Foundation has directly supported the following awards/honors, publications, and presentations:

Awards/Honors

Dr. Roehrl was awarded a Young Investigator Award from the Human Proteome Organization and the International Forum for Proteomics at the HUPO 2010 meeting in Sydney, Australia.
Dr. Roehrl has been invited to serve as a founding Associate Editor of the new Journal of Integrated OMICS.
Dr. Roehrl will serve as course director of two new postgraduate courses on Systems Pathology and Omic Approaches in Modern Personalized Pathology for the United States States and Canadian Academy of Pathology and the American Society of Clinical Pathology.

Peer-reviewed papers

Wang SS, Wang JY, Roehrl MHA. Discovery of colon adenocarcinoma metabolomes via 1H NMR spectroscopy. Submitted.
Rho JH, Zhang W, Murali M, Leone S, Roehrl MHA*, Wang JY*. American Journal of Pathology 2011; 178: 2177- 2190. *Corresponding authors.
Wang JY, Lee J, Yan M, Rho JH, Roehrl MHA. American Journal of Pathology 2011; 178: 2168-2176.
Roehrl MHA*, Alexander MP, Hammond SB, Ruzinova M, Wang JY, O'Hara CJ. American Journal of Hematology 2011 (in press). *Corresponding author.
Toribio Y, Roehrl MHA. Archives of Pathology and Laboratory Medicine 2011 (in press).
Roehrl MHA*, Wang JY. American Journal of Hematology 2011; 86: 307-308. *Corresponding author.
Roehrl MHA*, Lantz D, Sylvester C, Wang JY. Archives of Pathology and Laboratory Medicine 2011; 135: 471-477.
*Corresponding author.

Book chapters in press

Roehrl MHA, Wang JY. Personalized Pathology: Tissue-Based Proteomics and Cancer Biomarkers. In: Proteomics.Rijeka, Croatia: InTech Publishing 2011. ISBN 978-953-307-832-8.
Roehrl MHA, Nielsen GP. Bone Lesions: Malignant Neoplasms. In: Molecular Pathology of the Head and Neck (Hunt JF, ed.). New York, NY: Springer 2011.
Roehrl MHA, Wang JY. Cancer Stem Cells. In: The Molecular Basis of Human Cancer, 2nd ed. (Coleman WB, Tsongalis GJ, eds.). New York, NY: Springer 2011.

Abstracts

Roehrl MHA, Wang SS, Wang JY. Laboratory Investigation 2011; 91 (S1): 446A.

Watts LYA, Alexander M, Wu H, Hammond S, Downey K, Toribio Y, Roehrl MHA. Laboratory Investigation 2011; 91 (S1): 133A.

Roehrl MHA, Rho JH, Wang JY. Laboratory Investigation 2010; 90 (S1): 427A.

Invited scientific presentations (2010-2011)

United States and Canadian Academy of Pathology, Association of Pathology Chairs, Human Proteome Organization, National Cancer Institute (Biospecimen Research Network);

Memorial Sloan-Kettering Cancer Center, Fred Hutchinson Cancer Research Center

1. Cancer Stem Cells

We have made progress in isolating CD133+ putative cancer stem cells from primary human colorectal cancer tissues using a combination of enzymatic tissue digestion and physical separation to remove stromal components from the cancer cells (Fig. 1).

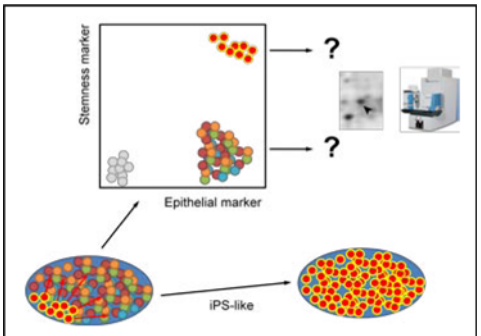


Figure 1. Cancer stem cell separation of primary human cancer specimens.

Currently, we are refining and scaling up these techniques to collect a sufficient cell mass to perform mass spectrometric proteomic analyses of the enriched cell populations.

In addition, I am the new Scientific Director of the BARC Biospecimen BioBank at Boston Medical Center and BUSM. In this new role, I have been able to completely redesign and innovate the way how we collect human biospecimens for cancer research. We have now implemented a system of "ultra-rapid" intraoperative biobanking (achieving a record <5 min ex vivo time) that allows unprecedented faithful preservation of the human pathophysiome within the tissue specimen and is key for proteomic discovery of disease-relevant proteins (Fig. 2).

Preliminary data is has been generated from proteomic analysis of $CD133^{hi}$ and $CD133^{lo}$ cell fractions (Fig. 3).

Support from the Grunebaum Foundation has been instrumental for both setting up the CD133 platform as well as implementing the novel tissue procurement workflows.

Boston University *(continued from previous page)*

2. Metabolomics

Colorectal cancer remains among the top causes of cancer mortality. Metabolomics, the comprehensive global characterization of small molecules involved in cellular biochemical transformations, is a powerful epigenetic approach to understanding the functional pathobiology of cancer and its underlying metabolic aberrations. High resolution liquid proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy is a sophisticated biophysical technique that allows a global, sensitive, and non-destructive interrogation of metabolomes extracted from human tissues (Fig. 4).

40 paired samples of fresh cancerous and adjacent normal colonic tissues were chemically extracted by a methanol-chloroform-water mixture. Tissue metablomes were partitioned into aqueous and organic phases and subsequently examined by high-resolution ^{1}H and ^{1}H-^{13}C NMR (Fig. 5). Fourier-transformed spectra were binned, normalized, and analyzed for statistically significant differences between normal and cancer groups. Principal component analysis (PCA) and partial least squares (PLS) discriminant analysis were used to distinguish normal spectra from cancer and to develop cancer biomarker models.

PCA and PLS discriminant analysis highly successfully distinguished normal spectra from cancer (Fig. 6). Studies of organic spectra indicated that cholesterol, cholesterol esters, and phospholipids were elevated in cancer samples, while triacylglycerol levels were depressed. Poly-unsaturated lipids also appeared predominantly in tumor biopsies, whereas mono-unsaturated lipids were more common in normal colon tissue. Analysis of aqueous samples showed increased quantities of uridine diphosphate N-acetyl-D- glucosamine, uracil, proline, lactate, O-phosphoethanolamine, glutamate, aspartate, oxidized glutathione, inosine monophosphate, and taurine in cancer spectra. In contrast, the metabolites glucose, myo-inositol, creatine, and scyllo-inositol were more prominent in normal spectra.

We discovered novel quantitative and qualitative metabolite biomarkers of human colorectal cancer. Using bioinformatics to map metabolomic differences onto network models of cellular metabolism, intriguing connections were discovered that hint at concerted large-scale metabolic readjustments in tumors and that provide exciting new directions for diagnostic test and drug target development. We show that metabolomics is a powerful and promising novel experimental approach and adds to the toolbox of systems pathology of disease.

Support from the Grunebaum Foundation has been instrumental for NMR spectroscopic data acquisition and computational data analysis.

Initial papers describing the above results have already been submitted.

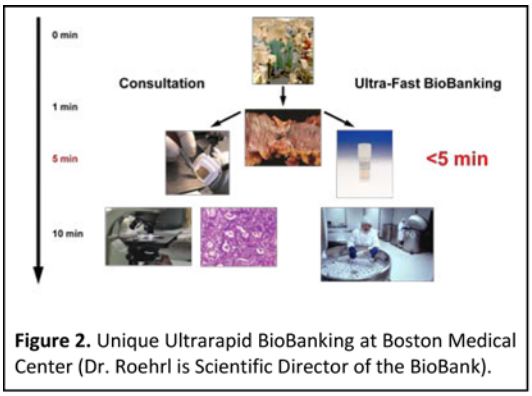


Figure 2. Unique Ultrarapid BioBanking at Boston Medical Center (Dr. Roehrl is Scientific Director of the BioBank).

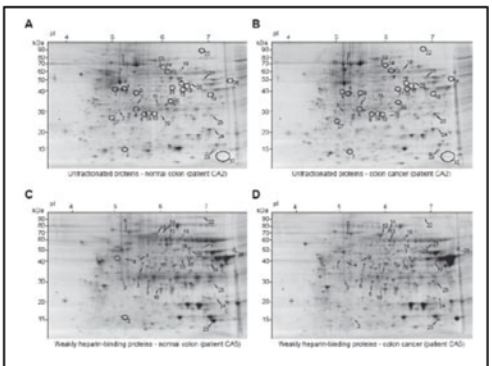


Figure 3. Preliminary 2-D gel electrophoresis results of the cancer stem cell proteome.

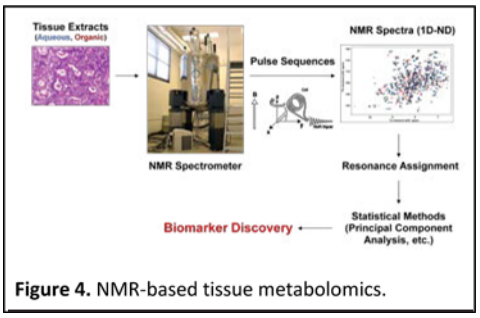


Figure 4. NMR-based tissue metabolomics.

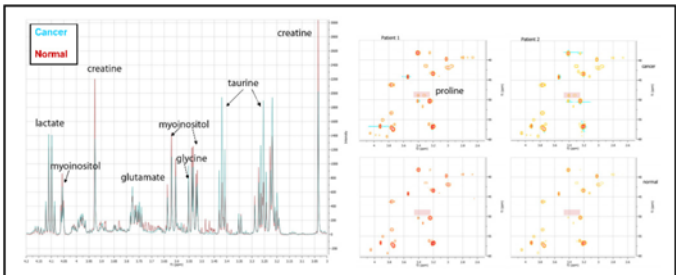


Figure 5. The human metabolome as seen by ^{1}H (left) and 2-D ^{1}H-^{13}C correlation (right) NMR spectroscopy.

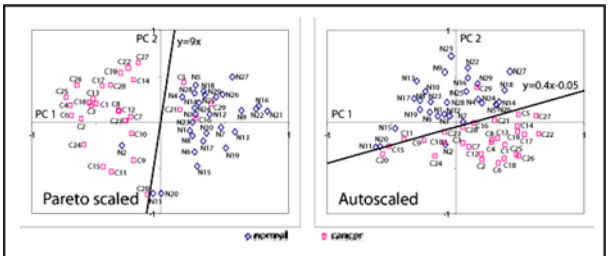


Figure 6. NMR metablomics distinguishes powerfully between normal (blue) and cancerous tissue (red).

Boston University School of Medicine

Synergistic Type Growth Inhibition in Breast Cancer Cells using HDAC Inhibitors and Capain Inhibitor in Combination

Mckenna Longacre: Post-Baccalaureate Student,
Summer Research Student, Cancer Center
Mentor: Dr. Sibaji Sarkar

As was previously mentioned in the Chair's letter, the Karin Grunebaum Cancer Research Foundation was able to donate funds to Harvard Medical School and the Boston University School of Medicine in order to support two very worthwhile programs. Funds were given to the Cancer Biology Area of Concentration Biological and Biomedical Services Ph.D. Program which were used for the Fall 2011 Welcome Event, the Dana Farber Cancer Institute Lunch Speaker Series and the Student Data Club. Funds were also given to two Boston University undergraduate students to try their hand at cancer research in a laboratory for the summer. The following is an overview of one of the programs:

Dr. Sibaji Sarkar's laboratory is developing a combination therapy for breast and ovarian cancers using histone deacetylase inhibitors (HDACi) and a protease calpain inhibitor. Calpain is a ubiquitious protease which regulates many signaling molecules. HDACi, including SAHA, are currently being used in clinical trials in combination with other cytotoxic drugs for various types of cancers, and have shown success in the treatment of lymphomas. HDACi is conventionally known to act by increasing acetylation. Our laboratory recently determined that HDACi demthylated CpG islands in the upstream promoter regions of silenced tumor suppressor genes by modulating ERK kinase. This finding has led us to investigate the mechanism of the combination treatment of HDACi with calpeptin in breast and ovarian cancer cells. We hypothesize that cancer cells are sensitized by HDACi and the combination of HDACi and calpeptin then induce apoptosis.

In continuation of previous work produced by our lab, this summer we investigated two different breast cancer cell lines: MCF7 (ER+, PR+, Her-2+) and MDA231 (triple negative), which is highly metastatic. There is a great need for effective treatments for triple negative breast cancers in particular, which currently account for 30% of total breast cancer. When used in suboptimal doses appropriate to each cell line, HDACi and calpeptin produced synergistic type growth inhibition in both cell lines. Cell cycle analysis by Propidium Iodide staining revealed significant inhibition in S phase compared to controls in the combination treatments. In addition, we observed decreased cell motility when these breast cancer cells were treated with HDACi and calpeptin. My current research includes investigation of both the mechanism of decreased cell motility in breast cancer cells, as well as the status of expression of cell adhesion molecules which are relevant in cell-cell attachment and motility.

IN THE SPIRIT OF THE SEASON....

Recently the KGCRF received the following note from our web designer, Greg Corwin, Owner of Image Genesis. We thought the note so heartwarming that we wanted to share it with you!

In a tradition that began over 25 years ago my siblings and I have had a Secret Santa gift exchange in lieu of getting gifts for each other. In recent years, with the addition of spouses and extended families, we change it so that every year a member of our family chooses a charity and makes a donationinstead.

This year it is my turn and in light of my mother's recent diagnosis and treatment of breast cancer, I have made the decision to choose KGCRF. The amounts are voluntary according to the individual finances of my four siblings and my parents, but I am also planning on donating one years' hosting on top of the cash donation. The final amount will be determined as the year ends, but I am shooting for $1000, including the hosting.

Happy Holidays to and your family!

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: **KGCRF, 85 Sherman Street, #8, Cambridge, MA 02140**

Online Support via PayPal at www.grunebaumfoundation.org