# Karin Grunebaum Cancer Research Foundation

October, 2007 • Volume 4 Founded in 1958



Dear Friends of the Karin Grunebaum Cancer Research Foundation:

It is hard to believe that the Foundation is approaching its 50th Anniversary. As many of you know, the Foundation was started when Karin Grunebaum died of cancer in June 1958. Since that time, the Foundation's mission has been to continually search for the best ways to help fight the battle against cancer. Initially (before the computer era), this involved funding a manual cancer registry at Salem Hospital so that physicians could compare their cancer patients' symptoms and treatment success with those of other patients who had preceded them.

Although this program was certainly beneficial to the concerned doctors and patients, the Trustees eventually decided that the Foundation's assets could more effectively be utilized by "investing in people" by funding one year of cancer-related research by medical students in hopes of encouraging them to eventually pursue careers in cancer research. Thus, in 1966, the Foundation began to fund medical student researchers at Harvard Medical School and also at Boston University School of Medicine starting in 1984.

In October 2006, the Trustees reviewed the program and determined that as we approach the Foundation's Silver Anniversary, our mission could more effectively be achieved by investing in people who had already made their career decision to work directly in cancer research. Accordingly, the Trustees decided that henceforth funding for one year of cancer-related research would be directed to faculty members at Harvard Medical School and Boston University School of Medicine who are within their first 5 years of appointment in laboratory or translational research in cancer. Such funding could be extended for a second year by competitive application of the researcher. Additionally, the Foundation will provide funds to help the Karin Grunebaum Faculty Research Fellows travel to seminars related to their research so that they can interact with the world's leading cancer researchers in their field.

We are thrilled with this new direction of growth, and are confident that this new path will prove even more effective in winning the cancer battle.

During the past year the Foundation was also gratified to be listed by *Guidestar.org* – the leading organization dedicated to informing the public about legitimate charitable organizations. We are hopeful that this listing will further encourage donations to our cause.

Our plans for the upcoming 50th Anniversary include development and continual updating of a dedicated website for the Foundation which will in part commemorate all the past Fellows and highlight their research projects and accomplishments. Obviously, this will cost money to develop and maintain. I am therefore asking each prior Karin Grunebaum Cancer Research Fellow to help fund not only this project but the Foundation itself to allow your Foundation to continue its mission for the next 50 years. As each of you received funding from the Foundation during your student years to help you achieve your personal goals and dreams, **I am personally asking you to please help the Foundation help your successors achieve their goals and dreams by donating at least $100 for each year since you were awarded a Karin Grunebaum Cancer Research Fellowship.** Naturally, we would also greatly appreciate donations from any other supporter.

Tax deductible contributions can be made to: Karin Grunebaum Cancer Research Foundation, 85 Sherman Street #8, Cambridge, MA 02140. Should you have any questions about the Foundation, please feel free to contact me directly.

Thank you.

Steven Wallach, Chairperson
Phone: (561) 750-7366; e-mail: wallachs@bellsouth.net

# CK2 Regulation Of Wnt Signaling In Breast Cancer and Development

**Isabel Dominguez, Ph.D., Research Assistant Professor**
**Section of Hematology-Oncology, Department of Medicine**
**Boston University School of Medicine**

Dr. Isabel Dominguez obtained her undergraduate and graduate degrees from prestigious Universities in Spain. With the support of two highly competitive research fellowships from the Spanish Ministry of Education and Science and the Basque government, she carried out excellent and productive post-doctoral research at Beth Israel Hospital and the Dana Farber Cancer Institute, where she began her studies of Wnt signaling in development and cancer. She was recruited to the Section of Hematology-Oncology at Boston University Medical Center in 2001 as an Instructor in Medicine to continue this line of research. This has led to highly productive collaborations and multiple publications in top journals, and to promotion to Research Assistant Professor in Medicine in 2003. Dr. Dominguez has authored 21 peer-reviewed articles and one review article in the Wnt field, has given many talks and presentations on her research at international meetings and at other universities, and teaches on Wnt signaling in advanced courses in the Biochemistry Department at Boston University School of Medicine.

Dr. Dominguez's main research interests focus on the characterization of cellular signaling pathways that are turned on transiently during normally embryonic development to allow cells to communicate with each other, and on how these signaling pathways can be abnormally reactivated and co-opted by tumor cells to gain aberrant growth advantage. Her interests in tumorigenesis started during her Ph.D. studies where she used the frog oocyte system to explore the mechanism of action of two signaling molecules, rasp21 and PKCz. These molecules are normally required to convert extracellular stimuli into growth signals inside the cell, and elevated activities of these molecules have been associated with oncogenic growth in many types of cancers. Both of these signaling molecules are established molecular targets for cancer therapeutics and pharmaceutical companies are evaluating the anti-tumor potential of inhibitors targeted against these molecules and their signaling pathways. In her postdoctoral research, she moved to studies of the canonical Wnt signaling pathway and she is currently dissecting the molecular components contributing to the oncogenic potential of aberrant Wnt signaling.

The canonical Wnt signaling pathway plays a key role during normal embryonic development; however, abnormally activity of this pathway is found in a number of cancers and is known to be the first one of the first molecular changes found in colorectal cancer. Dysregulation of the Wnt pathway can lead to transactivation of oncogenic factors such as cyclin D1, c-myc, and matrix metalloproteinases and can render the tumor cells with additional growth benefits. Dr. Dominguez's current interest is to understand kinase regulation of the Wnt pathway in development and cancer. Her strategy is to utilize embryos from the frog *Xenopus laevis* to understand basic biochemical and molecular mechanisms of Wnt signaling and apply this knowledge to the study of cancer. In frog embryos, canonical Wnt signaling components can be easily, rapidly and efficiently assayed. For example, overexpression of various Wnt pathway components in the ventral side of the fertilized egg can cause embryos to form a duplicated body axis, *i.e.* the embryos have two backs and two heads ("ectopic axis assay", Fig. 1). Using this specific functional assay and biochemical assays, she was the first to elucidate how the serine/threonine protein kinase GSK3b is regulated in Wnt signaling during early frog embryonic development. Using this assay, she has also shown that another protein kinase called protein kinase CK2 can mimic Wnt signaling (Fig. 2) and it is involved in early frog development. Interestingly, CK2 is highly expressed in many human cancers. Ectopic expression of CK2 in mouse breast tissue and lymphocytes can lead to formation of breast and lymphoid tumors. Furthermore, CK2 and the canonical Wnt pathway are activated in mouse mammary tumors induced by environmental carcinogens.

*Figure 1. Wnt expression leads to body axis duplication*

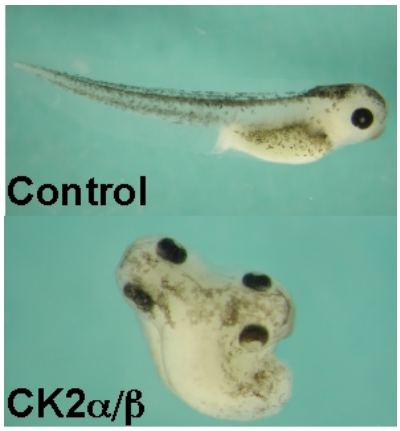

*Figure 2. CK2 is sufficient to induce ectopic axis*

To further understand the role of CK2 in tumorigenesis, Dr. Dominguez has shown that CK2 regulates the stability and function of b-catenin, a key component of canonical Wnt signaling that is found mutated and excessively activated in human tumors, especially colorectal tumors. One of Dr. Dominguez's goals is to develop novel inhibitors of CK2 that can block Wnt-dependent biological processes including aberrant signaling in transformed cells, and to use these inhibitors to validate CK2 as a good molecular target for anti-tumor therapeutics.

# Report from Doctor Ferrone

**Christina R. Ferrone, M.D., Assistant Residency Program Director, Massachusetts General Hospital**

American Cancer Society predicts 37,170 new cases and 33,370 deaths from pancreatic adenocarcinoma in 2007[1]. Less than 15% of patients diagnosed with pancreatic adenocarcinoma will undergo surgical resection. Despite extensive research for adjuvant therapy, surgical resection remains the only potential cure. The ability of a host to mount an effective immune response against cancer has offered hope for many diseases that still lack durable, effective, and non-toxic treatments, including pancreatic adenocarcinoma. Tumor infiltrating lymphocytes in melanoma[2], colorectal cancers[3-5], and ovarian cancer[6,7], have shown to inhibit tumor growth and are associated with improved prognoses. Our first aim is 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 recent revival of interest in the role of immunosurveillance in the control of tumor growth has provided the impetus to develop and implement immunotherapeutic strategies for the treatment of malignant diseases[8]. These strategies have greatly benefited from the availability of well-characterized human tumor antigens (TA) and from the major progress made in our understanding of the molecular interactions, which lead to the development of a TA-specific immune response[9,10]. Despite the implementation of a large number of clinical trials at many institutions with large numbers of patients, the clinical results have been rather disappointing[11]. This may be due to a lack of significant correlation between clinical and immunological responses. This disappointing discrepancy is likely to reflect, at least in part, the ability of tumor cells to develop escape mechanisms, which allow them to avoid recognition and destruction by a host's immune system[12,13]. The escape mechanisms which have been identified include: defects in the development of a TA-specific immune response, inability of immune cells to migrate and infiltrate malignant lesions, abnormalities in the recognition of tumor cells by immune cells, development of resistance mechanisms in tumor cells to lysis by immune cells, and the release of immunosuppressive agents from tumor cells to inactivate immune cells[12,13].

Little information is available regarding the potential immune escape mechanisms in pancreas carcinoma. Understanding immune escape would contribute to the design of immunotherapeutic strategies for the treatment of pancreas carcinoma. The long-range goal of our research program is to characterize the mechanisms of immune escape and develop approaches to counteract these mechanisms utilized by pancreas adenocarcinoma cells. This project would focus on the immune escape mechanism involving the shedding of NKG2D binding molecules by pancreatic cancer cells. Shedding of NKG2D binding molecules is associated with down regulation of its receptor on CD8ab T cells and NK cells, thereby impairing lysis of tumor cells[14]. Specifically, we would investigate the levels of soluble MHC class I chain related protein A (MICA) and MHC class I chain related protein B (MIBC), which have been documented to influence the clinical outcome of patients with various types of cancers[15,16]. Interestingly, development of high titer anti-MICA-specific antibodies is associated with an improvement in the clinical course of patients with metastatic melanoma who have been vaccinated with lethally irradiated tumor cells engineered to secrete granulocyte-macrophage colony stimulating factor[15]. The MICA-specific antibodies opsonize tumor cells for dendritic cell cross-presentation and in conjunction with complement lyse malignant cells. We have evaluated patients with pancreatic adenocarcinoma who develop anti-MICA-specific antibodies to determine if they have an improved survival relative to the patients who do not develop anti-MICA antibodies.

In summary, the specific aims will test the hypothesis that:
i.) Quantity and type of immune infiltrate (cytotoxic T cells, helper T cells, regulatory T cells, memory T cells, NK cells) affects the tumor's metastatic potential and therefore a patient's clinic outcome.
ii.) Serum MICA and MICB levels correlate with the extent of lymphoid cell infiltrate in malignant lesions, and therefore with the clinical course of the disease, as measured by recurrence free survival and overall survival.
iii.) Development of high titer anti-MICA-specific antibodies is associated with an improvement in the clinical course of the disease and with an enhancement of lymphoid cell infiltrates in malignant lesions.

The information derived from the outlined studies will contribute to our understanding of the mechanisms underlying the inability of a host's immune system to control tumor growth and may suggest targeted strategies to counteract escape mechanisms utilized by tumor cells in patients with pancreas carcinoma.

## References

1.American Cancer Society. Facts and Figures 2006. Atlanta, GA, American Cancer Society, 2007
2.Clemente CG, Mihm MC, Jr., Bufalino R, et al: Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 77:1303-10, 1996
3.Baier PK, Wimmenauer S, Hirsch T, et al: Analysis of the T cell receptor variability of tumor-infiltrating lymphocytes in colorectal carcinomas. Tumour Biol 19:205-12, 1998
4.Diederichsen AC, Hjelmborg JB, Christensen PB, et al: Prognostic value of the CD4+/CD8+ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLA-DR expression on tumour cells. Cancer Immunol Immunother 52:423-8, 2003
5.Naito Y, Saito K, Shiiba K, et al: CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res 58:3491-4, 1998
6.Sato E, Olson SH, Ahn J, et al: Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A 102:18538-43, 2005
7.Zhang L, Conejo-Garcia JR, Katsaros D, et al: Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 348:203-13, 2003
8.Dunn GP, Old LJ, Schreiber RD: The immunobiology of cancer immunosurveillance and immunoediting. Immunity 21:137-48, 2004
9.Novellino L, Castelli C, Parmiani G: A listing of human tumor antigens recognized by T cells: March 2004 update. Cancer Immunol Immunother 54:187-207, 2005
10.Parmiani G, Castelli C, Dalerba P, et al: Cancer immunotherapy with peptide-based vaccines: what have we achieved? Where are we going? J Natl Cancer Inst 94:805-18, 2002
11.Rosenberg SA, Yang JC, Restifo NP: Cancer immunotherapy: moving beyond current vaccines. Nat Med 10:909-15, 2004
12.Whiteside TL: Immune suppression in cancer: effects on immune cells, mechanisms and future therapeutic intervention. Semin Cancer Biol 16:3-15, 2006
13.Marincola FM, Jaffee EM, Hicklin DJ, et al: Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol 74:181-273, 2000
14.Wiemann K, Mittrucker HW, Feger U, et al: Systemic NKG2D down-regulation impairs NK and CD8 T cell responses in vivo. J Immunol 175:720-9, 2005
15.Jinushi M, Hodi FS, Dranoff G: Therapy-induced antibodies to MHC class I chain-related protein A antagonize immune suppression and stimulate antitumor cytotoxicity. Proc Natl Acad Sci U S A 103:9190-5, 2006
16.Osaki T, Saito H, Yoshikawa T, et al: Decreased NKG2D expression on CD8+ T cell is involved in immune evasion in patients with gastric cancer. Clin Cancer Res 13:382-7, 2007

# Pancreatic Cancer Tumor Response to Hedgehog Pathway Inhibition in a Xenograft Model

**Stefan Fritz, M.D.**
**Massachusetts General Hospital and Harvard Medical School**
**Advisor: Sarah P. Thayer, M.D., Ph.D.**

**Introduction:** Pancreatic adenocarcinoma is among the most lethal of human cancers, typically presenting as advanced inoperable disease with rapidly progressive clinical course characterized by intense resistance to all therapeutic modalities. One of the new targets for treatment of pancreatic adenocarcinoma recently identified is the Sonic Hedgehog (Shh) pathway. A previous pilot study revealed that inhibition of the Hh pathway suppresses the growth of these pancreatic tumors. One resulting hypothesis was that tumor differentiation may be a predictor of tumor response to Shh pathway inhibition. In this present study we expand the number of xenograft lines to cover all grades of tumor differentiation and increase the number of treatment mice to determine if Shh remains an effective therapeutic treatment and to evaluate if there are response differences in between the grades of tumor differentiation.

**Methods:** Xenografts were created from 7 patient-derived pancreatic adenocarcinoma tissue samples with grades of differentiation varying from well-to-moderately to poorly differentiated. Mice with tumors e"125mm$^3$ were randomly divided into three treatment groups of 5 animals each. Mice were treated with Hh pathway inhibitors 5E1 (anti-Shh antibody), cyclopamine (Smoothened inhibitor) and forskolin (Gli-antagonist) over the course of seven days; a control group received normal saline. Treatment response was determined by tumor volume change, proliferation index (IHC, Ki67-staining) and percentage of viable glands. Furthermore real time RT-PCR was performed for quantification of Hh pathway members (Ptch1, Ptch2, Gli1, Gli2) before and after treatment.

**Results:** 100 mice from 7 patient lines were studied and treated. All tumors misexpress Hh and have an active Hh pathway. Pathway activity was determined by the upregulation of downstream effectors Ptch1, Ptch2, Gli1 and Gli2. After treatment all tumors showed marked decrease in Hh pathway activity as assessed by the decreased expression of at least two of four target genes (Ptch1, Ptch2, Gli1, Gli2).

Moderately differentiated tumors (n=33) showed a significant reduction of tumor growth by 93.17% (p=0.03), a significant reduction of viable gland density of 47.58% (p=0.005) and a significant reduction of the proliferation index by 67.25% (p=0.045). Moderately to poorly differentiated tumors (n=20) showed a reduction of tumor growth of 96.02%, a significant reduction of Ki67 positive cells by 56.22% (p=0.021) and a significant reduction of viable gland density of 46.89% (p=0.004).

Poorly differentiated tumors (n=21) showed a decrease of tumor growth by 99.49% (p=0.107), but no significant differences were seen in proliferation index or in viable gland density. No significant differences were seen between the different treatment groups (5E1, cyclopamine and forskolin).

**Conclusions:** Our xenograft model reflects the wide range of patho-biological features and genetic alterations that characterize human pancreatic cancers. In this present study, Shh pathway inhibition is an effective treatment option in a subset of human pancreatic cancers. This study supports our hypothesis that one strong predictor of anti-tumor response to Shh pathway inhibition appears to be tumor differentiation. Poorly differentiated tumors may partially respond by changes in volume, but these tumors appear to be independent of the Hh pathway for proliferation and survival.

### A promising new pancreatic cancer tumor treatment?

***Today, pancreatic cancer remains a lethal disease, most patients die within five years after diagnosis. Presently, there is no effective chemotherapy available.***
***Our research group has recently identified a novel therapeutic target called Sonic Hedgehog that may be effective in killing pancreatic cancer cells. Under normal conditions Sonic Hedgehog is an important developmental molecule. Our laboratory has discovered that this molecule is abnormally present in pancreatic cancer and the associated tumor pathway plays an important role in cancer cell survival.***
***In our current experiments we found that inhibition of this pathway results in death of pancreatic tumors derived from patients who recently have undergone surgery. Our findings reveal that this pathway might be a novel therapeutic target for treatment of pancreatic carcinoma in the near future.***

## About Stefan Fritz, M.D.

Stefan Fritz, M.D. graduated from medical school at the University of Heidelberg, Germany in 2003. During his medical studies, he performed a number of foreign clinical clerkships (Johannesburg, Chapel Hill, Paris) and completed his dissertation for the medical degree entitled “Conditioning of liver donors by Kupffer cell blockade – Effects on liver, heart, lung and kidney.”

After medical school, he began his surgical residency at Heidelberg University. As part of the European Pancreas Center under Dr. Buchler, his research initially focused on acute pancreatitis. During three years of residency, he also participated in one year at the National Center for Tumor Diseases in Heidelberg, where he gained practical and theoretical knowledge about pancreatic diseases.

To improve his research skills and broaden his knowledge about pancreatic disease, Dr. Fritz became a research fellow in Dr. Sarah P. Thayer’s Pancreatic Biology Research Laboratory at the Massachusetts General Hospital. During his two years of research, he is working on two main topics: xenograft modeling to evaluate the efficacy of a new treatment target for pancreatic cancer and detection of chromosomal aberrations in intraductal papillary mucinous neoplasms (IPMNs), a relatively rare and recently described pancreatic tumor type.

After his research fellowship in Boston, Dr. Fritz will return to Germany to continue his academic career in surgery. His major interests are General and Visceral Surgery with focus on pancreaticobiliary surgery and translational pancreatic cancer research.