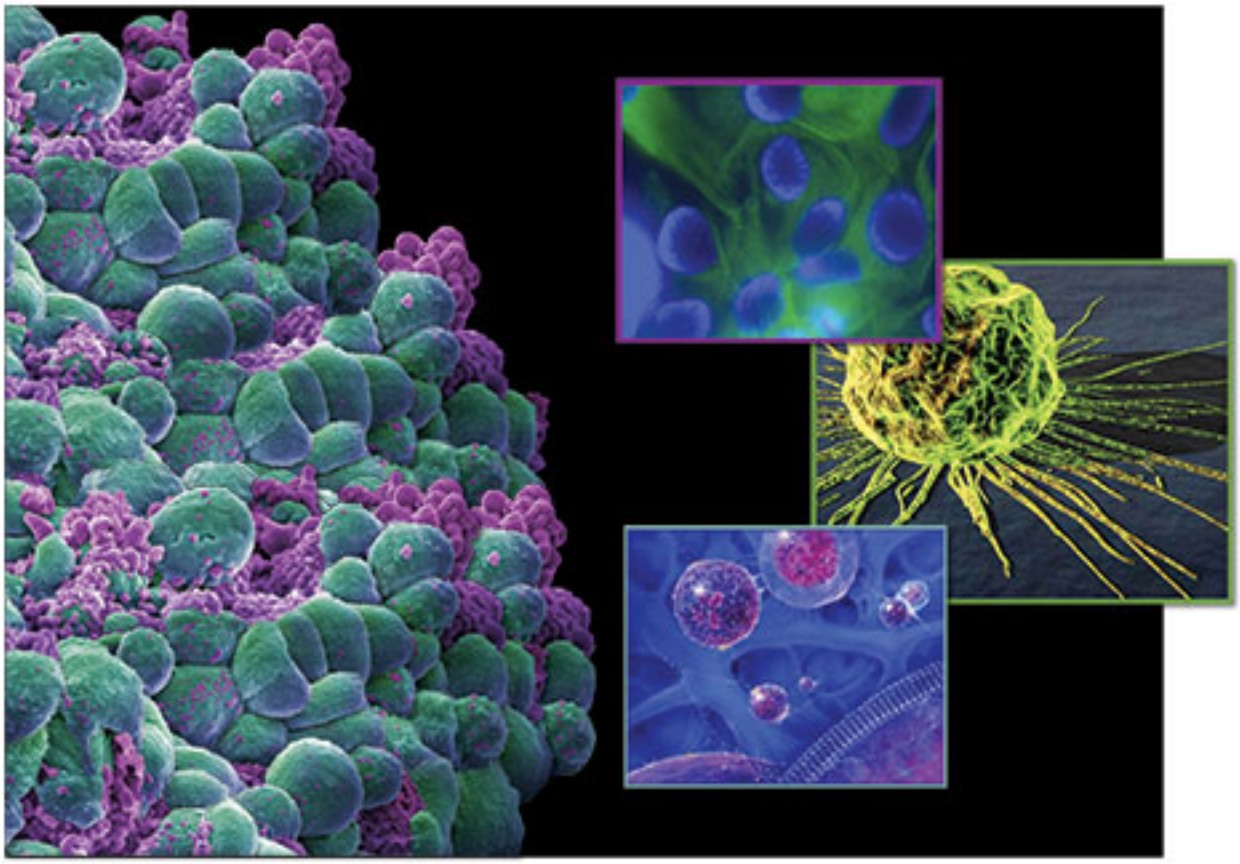
Tufts University

Research Days 2008 – 2009

Cancer Research

Poster Presentations



Friday, October 17, 2008

Sponsored by the Office of the Vice Provost

Title:

Is the Mammary Stroma of Copenhagen Rats a Tumor Suppressor?

Authors:

Amorette Drexler, Cheryl Schaeberle, Maricel Maffini

Presented by:

Maricel Maffini

Department:

Department of Anatomy and Cellular Biology, Tufts University School of Medicine

Abstract:

Copenhagen (COP) is a rat strain that does not develop tumors when exposed to chemical carcinogens such as N-nitrosomethylurea (NMU) during the window of vulnerability of 50-60 days of age commonly used in cancer research. However, comparative studies showed that COP rats develop a similar number of preneoplastic lesions as the tumor-susceptible Wistar-Furth (WF) rats. While in the COP rats, these lesions regress those in the WF rats progress and develop into carcinomas. The resistance phenotype was attributed to the presence of mammary carcinoma susceptibility genes in COP mammary epithelium and their absence in WF rats, although contradictory results have been published. Previously, we showed that, on one hand, NMU-exposed WF mammary stroma is able to induce normal mammary epithelial cells (MEC) to form carcinomas. On the other hand, normal WF mammary stroma has also the ability to reverse the tumor phenotype of malignant epithelial cells and induce them to form normal mammary ducts. In light of these results, we postulated that the stroma is also responsible for the tumor-resistant phenotype observed in the COP rats.

The experimental design was 2-fold:

1) Tissue Recombination: We recombined stromal and epithelial cells from WF and COP rats; mammary fibroblasts (MF) were collected from cleared-fat pads of animals exposed to NMU or vehicle (VEH). MECs were collected from virgin 55 day-old rats. The experimental groups were:

Group 1: MF from NMU-exposed COP rats were recombined with COP MEC (positive control for tumor resistance)

Group 2: MF from VEH-exposed COP rats with COP MEC

Group 3: MF from NMU-exposed COP rats with WF MEC

Group 4: MF from VEH-exposed COP rats with WF MEC

Group 5: MF from NMU-exposed WF rats with WF MEC (positive control for tumor susceptibility)

Group 6: MF from VEH-exposed WF rats with WF MEC

Group 7: MF from NMU-exposed WF rats with COP MEC

Group 8: MF from VEH-exposed WF rats with COP MEC

Both cell types were mixed with collagen I and grafted under the kidney capsule of host SCID mice. All tissue recombinants developed normal ducts; however, no recombinants developed macroscopic tumors. Preneoplastic and neoplastic lesions were only observed in Groups 5 and 7 (NMU-exposed WF fibroblasts). Groups 1 and 3 (NMU-exposed COP fibroblasts) did not show any neoplastic or preneoplastic lesions. Instead, most structures were normal ducts. These findings suggest that the tumor resistant phenotype observed in COP rats resides in the mammary stroma.

2) Global Gene Expression Analysis: COP and WF mammary stroma were collected from NMU- or VEH-exposed animals after 15 (COP and WF have equal number of preneoplastic lesions) or 60 (lesions regressed in COP and progressed in WF) days after treatment (DAT). Our data indicate that the amount of HOXA1 and STAT3 mRNAs is lower in the COP-NMU rats compared to the WF-NMU. STAT3 mRNA is lower at 5 and 15DAT while HOXA1 is lower at 15 and 60DAT. COP-NMU animals have an increase in TGF beta2 and procollagen type X mRNAs at 15DAT compared to WF-NMU. These and other genes expressed at 15DAT, when the animals showed the highest number of preneoplastic lesions, seem to be setting the stage for their disappearance by remodeling the stroma to reduce (or shut off) epithelial cell proliferation and to induce normalization of the lesions.

Title:

Stromal Influences on the MCF7 Cells Phenotype in 3-Dimensional Cultures

Authors:

Silva Krause, Maricel Maffini, Ana Soto, Carlos Sonnenschein

Presented by:

Maricel Maffini

Departments:

Department of Cell, Molecular and Developmental Biology and of Anatomy and Cellular Biology,
Tufts University School of Medicine

Abstract:

Stromal-epithelial interactions mediate mammary gland development and the formation and progression of breast cancer. In order to study these interactions in more detail, the development of defined 3-dimensional *in vitro* models is essential. In the present study, we have successfully developed novel 3-dimensional *in vitro* breast cancer models, which allow the study of both tumor reversion and tumor formation. Co-cultures of a human breast cancer cell line MCF7 and human mammary fibroblasts obtained from reduction mammoplasties embedded in either a type I collagen or a mixed Matrigel™-collagen matrix were carried out for up to 6 weeks. Histological and ultrastructural analysis confirmed the formation of epithelial structures. The importance of the stromal cells was apparent in both matrices; in the collagen gels the presence of reduction mammoplasty fibroblasts reduced epithelial cell apoptosis and allowed them to become polarized before obtaining tumor-like structures and in the mixed Matrigel™-collagen gels the presence of those fibroblasts initially resulted in the reversion of the neoplastic phenotype and later on, these epithelial structures formed tumor-like structures. These models provide an excellent system to study tissue organization, epithelial morphogenesis and breast carcinogenesis.

Title:

In Utero Exposure to Bisphenol A: Links with Mammary Gland Cancer Development

Authors:

Tessa Murray, Maricel Maffini, Carlos Sonnenschein, Ana Soto

Presented by:

Tessa Murray

Department:

Department of Anatomy and Cellular Biology, Tufts University School of Medicine

Abstract:

Epidemiological studies suggest that fluctuating estrogen levels in the fetal environment have long-term consequences regarding the risk of developing breast cancer during adult life. In addition, experimental studies showed that perinatal exposure to pharmacological doses of diethylstilbestrol (DES) increased the incidence and decreased the latency period of mammary cancer. We observed that fetal exposure to environmentally relevant doses of the xenoestrogen bisphenol A (BPA), a compound used in the manufacture of polycarbonate plastics, alters the normal development of the mouse mammary gland resulting in long-lasting effects manifested mainly during adult life. Of particular interest were: (i) an increase in the number of terminal end buds and terminal ends, (ii) an increase in ductal density (breast density is considered a risk factor), and (iii) an increased sensitivity to estradiol, suggesting enhanced susceptibility for mammary cancer development. Hence, we hypothesize that in utero exposure to low doses of BPA increases the risk of developing mammary cancer.

To explore this hypothesis, we used a Wistar-Furth rat model. Animals were exposed to BPA (2.5, 25, 250 or 1000µg/kg/day) or vehicle (50% DMSO) from gestational day 9 to birth using an osmotic pump. In utero, BPA exposure caused an increase in the incidence of preneoplastic lesions (ductal hyperplasias) at postnatal day 50, 95 and 140 regardless of the BPA dose. Interestingly, animals exposed to the lowest dose tested (2.5µg BPA/kg/day) showed a consistent and statistically significant increase in ductal hyperplasias relative to unexposed animals at all ages. More importantly, carcinomas in situ (CIS) were also observed in these animals. We postulate that the persistent ductal hyperplasias may develop into CIS later in life. To test this hypothesis we are currently analyzing tissue collected from animals at postnatal day 140 and 200. Also, we are conducting morphometric analyses at all four developmental stages to determine whether BPA exposure has caused additional long-lasting morphological changes in the mammary gland, especially paying attention to the terminal end buds as carcinomas are thought to originate in these structures.

In summary, in utero BPA exposure induces the development of preneoplastic and neoplastic lesions in the mammary gland in the absence of any additional treatment aimed at increasing tumor development. Our results support the hypothesis that environmental exposure to xenoestrogens during fetal life may contribute to the increased incidence of human breast cancer observed over the past 5 decades.

Title:

Parity Alters Responses to Ionizing Radiation in the Mammary Gland

Authors:

Matthew Carter, Melissa Troester, Melissa Johnson, Joseph Jerry, Sallie Smith Schneider

Presented by:

Matthew Carter

Department:

Pioneer Valley Life Sciences Institute, Baystate Medical Center

Abstract:

Parity is associated with a significant reduction in breast cancer risk. Identification of the key pathways altered by parity will provide novel molecular targets for the development of chemo-protective agents. Radiation is a potent carcinogen in the mammary gland and causes mutations through its ability to induce DNA damage, as well as through its ability to induce "bystander effects." These bystander effects have been shown to be tumor promoting in immortalized cells and they involve the modulation of various growth factors required for stem cell maintenance. We hypothesized that we could identify specific genes involved in parity-induced protection by examining gene expression responses of normal breast tissue from parous and nulliparous women.

Methods*:* Women undergoing elective reduction mammoplasties were asked to enroll in our study and donate their excised tissue. Small portions of this tissue were put into tissue culture for 24 hours and then exposed to 5Gy of radiation. Subsequently, the tissue was left in culture for an additional six hours to allow for uninterrupted changes in gene expression. Finally, the tissue was fixed for immunohistochemical analysis or RNA was harvested for microarray analysis.

Results: A SAM analysis revealed a loose 200 gene signature which showed differential transcriptional responses between parous and nulliparous women in response to radiation. Breast tissue harvested from nulliparous women showed the most striking changes with an increased expression of 80% of genes in response to radiation whereas tissue from parous women showed no change or decreased expression of these same genes. The other 20% of signature genes were strongly down-regulated in tissue from nulliparous women in response to radiation, while they were up-regulated or remained unchanged in parous tissue. Many of the genes which seem to be inversely affected by radiation indicate that permanent changes caused by parity involve pathways that regulate tissue homeostasis, wound repair, as well as DNA replication and repair. For instance, transforming growth factor-beta (TGF-β) is a multifunctional cytokine which regulates both tissue development as well as repair processes. The data obtained from the microarray indicated that in nulliparous women, the expression of the type III TGF-β receptor (TβRIII) is reduced in response to radiation. Reductions in the levels of TβRIII are interesting as it is down-regulated in a wide range of human cancers, including breast cancer, and re-expression can inhibit cancer progression. Our current work is aimed at verifying these microarray data utilizing quantitative real-time PCR analysis to measure mRNA expression levels and IHC to assess cellular protein levels. Taken together, these findings suggest a putative mechanism by which a full-term pregnancy conveys its protective effect against breast cancer.

Title:

Defining the Cellular Origins of Human Breast Cancer Heterogeneity

Authors:

Patricia Keller, Ina Klebba, Piyush Gupta, Hannah Gilmore, Stuart Schnitt, Charlotte Kuperwasser

Presented by:

Patricia Keller

Departments:

Department of Anatomy and Cellular Biology, Tufts University School of Medicine; Molecular Oncology Research Institute, Tufts Medical Center; Broad Institute of Massachusetts Institute of Technology and Harvard University; Department of Pathology, Beth Israel Deaconess Medical Center

Abstract:

Human breast cancers can be broadly classified based on their molecular and gene expression profiles into luminal and basal tumors. These tumor subtypes express markers corresponding to the two major differentiation states of epithelial cells in the breast; luminal cells that line the breast ducts and the outer myoepithelial/basal cells that provide contractile functions. Although there is likely a complex interplay of factors that contribute to tumor phenotype, the persistence of characteristics of normal breast cell types in tumors suggest that the major tumor subclasses could arise from different cells of origin. We have recently described an experimental model in which both the epithelium and stromal compartments are derived from human tissues. This model has enabled the creation of normal and neoplastic breast tissues *in vivo*. Breast cancers were created from single cell suspensions of human breast epithelial cells that were transformed and injected into humanized mammary fat pads. The epithelial cells were maintained *in vitro* for no more than 24 hours. Histological analysis revealed that the tumors were heterogeneous invasive carcinomas with features of both basal and luminal subtypes. To determine if different cells of origin influence the cancer phenotype, we enriched cells of the basal/myoepithelial lineage and cells of the luminal lineage by sorting freshly isolated human mammary epithelial cells for the markers CD10 and ESA, respectively. These enriched populations were infected and injected in the same fashion as unsorted cells. CD10+ basal cells formed well-circumscribed tumor nodules that exhibited increased basal differentiation. Tumors from the ESA+ luminal enriched fraction formed expansive ER+ invasive carcinomas containing reduced squamous differentiation, consistent with luminal differentiation. These results suggest that the cellular differentiation state of the cell of origin can persist in the resultant tumors. To determine if the genetic background of the breast tissue also influences the tumor phenotype, we transformed breast epithelial cells derived from patients who have had prophylactic mastectomies due to inherited BRCA1 mutations. Tumors that formed from unsorted BRCA1 cells exhibited increased basal/myoepithelial phenotype than those from non-BRCA1 patient samples. These results suggest that the association of basal-type tumors with BRCA1 breast cancers may result from an altered differentiation state in breast tissues of BRCA1 patients.

Title:

Morphology and Proliferation Control of Normal and Malignant Breast Epithelial Cells in a Collagen-Based 3-Dimensional Model of Tissue Microenvironment

Authors:

Eugen Dhimolea, Maricel Maffini, Ana Soto, Carlos Sonnenschein

Presented by:

Eugen Dhimolea

Department:

Department of Anatomy and Cellular Biology, Tufts University School of Medicine

Abstract:

In order to understand carcinogenesis and predict the outcome of pharmacological treatments, *in vitro* models that recapitulate the three-dimensional (3D) structural and functional context of normal and malignant tissues will be very informative. Surrogate models have provided significant insight on the morphology of normal and tumorigenic breast cells in 3D matrices. However, the cell-mediated re-organization of the Extracellular Matrix (ECM) has not been thoroughly investigated at the tissue level because the exclusive use of the epithelia cell type and estrogenic contamination in the tissue culture plastic labware prevent addressing important questions regarding the significance of interactions between epithelial and stromal cells in breast cancer and tumor response to hormone therapy, respectively.

We developed an estrogen-free 3D culture of normal and breast cancer cell lines and primary breast fibroblasts in collagen type I gels. A normal human breast epithelial cell line (MCF10A) contracts the collagen gels and increases their stiffness while acini- and ductal-like structures that resemble the morphology of normal mammary gland are formed. This happens independently of the presence of breast fibroblasts. Analysis of collagen fiber configuration by picrosirius red staining demonstrates a differential rearrangement of collagen around the cellular structures, similar to the fibers surrounding the normal gland epithelium *in vivo*. Impeding of gel contraction prevents the formation of organized structures by the MCF10A cells and results in random distribution of cellular aggregates. In contrast, the estrogen-sensitive breast cancer MCF-7 cells do not cause contraction of the collagen gel and form loose disorganized colonies. Interestingly, the combination of fluorescent protein-labeled MCF-7 and MCF10A induces contraction of collagen and formation of compact spherical MCF-7 structures that are different from the colonies in MCF-7 cells alone in culture. Given that circa 70% of breast tumors express the Estrogen Receptor, we tested the sensitivity of our model to estrogens. The MCF-7 cells retain the responsiveness to proliferative and anti-proliferative stimuli of estradiol and antiestrogens (OHT and fulvestrant) respectively, in 3D conditions. Thus, collagen type I gels are useful 3D *in vitro* models for studying the role that cell-cell and cell-ECM interactions and biophysical forces play on the morphology of normal and epithelial breast cancer cells. This model provides the opportunity for experimental hormonal manipulations aiming at elucidating the development of drug resistance mechanisms at the tissue organizational level in estrogen-dependent breast cancer.

Title:

Knockdown of Spry2 by RNA Interference in Human Mammary Epithelial Cells Results in Increased Cell Cycle Progression and Migration

Authors:

Robert Friesel, Jessica Toher, Lucy Liaw

Presented by:

Robert Friesel

Department:

Center for Molecular Medicine, Maine Medical Center Research Institute

Abstract:

Dysregulation of receptor tyrosine kinase (RTK) signaling is a major contributor to human carcinogenesis. Spry2 is a feedback antagonist of RTK signaling targeting components of the Ras-Raf-ERK pathway, a pathway known to be elevated in many types of cancer. Spry2 is down-regulated in a variety of human tumors, which may contribute to tumor progression and metastasis. Using an immortalized human breast cell line, MCF10A, as a model, we investigated the impact of the loss of Spry2 by RNA interference on the oncogenic transformation of MCF10A cells in vitro. Lentiviral vectors encoding Spry2-specific shRNAs were stably transduced into MCF10A cells. MCF10A cells, in which Spry2 was stably knocked down, exhibited loss of contact inhibition, increased cell growth, increased cell migration and a dramatic increase in the number of cells in S phase. In addition, there was an increase in β-catenin protein levels and redistribution away from the cell membrane. Overexpression of Spry2 in the MDA-MB-231 breast cancer cell line results in decreased cell growth and migration. Taken together, these data indicate that Spry2 plays an important role in the regulation of breast epithelial cell growth and supports the clinical observation that loss of Spry2 in human breast cancers contributes to the malignant phenotype.

Title:

The Wnt/β-Catenin Signaling Antagonist, SFRP1, Directly Affects the Tumorigenic Properties of Non-Malignant and Malignant Mammary Epithelial Cells

Authors:

Kelly Gauger, Jeremy Hugh, Jennifer Ostrander, Sallie Smith Schneider

Presented by:

Kelly Gauger

Departments:

Pioneer Valley Life Sciences Institute, Baystate Medical Center; Departments of Biology and of Veterinary and Animal Science, University of Massachusetts Amherst

Abstract:

Breast cancer is the most frequently occurring cancer in women and represents the second leading cause of cancer death among women. It is essential to understand the mechanistic actions by which breast cancer occurs and how it can be prevented. For example, aberrant activation of the Wnt/β-catenin signaling pathway contributes to the genesis of a wide range of human cancers, including breast cancer. Secreted frizzled-related proteins (SFRPs) are a family of proteins that antagonize the Wnt/β-catenin signaling pathway. Considering that the SFRP1 isoform is down-regulated in breast tumors, our hypothesis is that loss of SFRP1 expression will render mammary epithelial cells more susceptible to tumorigeneis *in vitro* and *in vivo*, and re-expression of SFRP1 into breast carcinoma cells will subsequently impede their cancerous characteristics. We have created a non-malignant immortalized mammary epithelial cell line (76N Tert) that stably expresses a siRNA construct that reduces the expression of SFRP1 by 80%. The phenotypic changes observed when SFRP1 alone is knocked-down in these cells, which include a loss of cell polarity causing a spindle-cell morphology and an increase in the formation of pseudopodia. Not only do these cells show signs of a more mesenchymal phenotype, but they also exhibit mesenchymal properities. Scratch-wound assays and matrigel assays revealed that SFRP1 loss in 76N Tert cells remarkably increases their ability to migrate and invade. Additionally, Wnt3a stimulation significantly increases proliferation as well as β-catenin-mediated luciferase activity when compared to empty vector transfected control cells. We have also generated a malignant breast cancer cell line (MDA-MB-231) that overexpresses SFRP1 and when these cells are stimulated with Wnt3a, β-catenin-mediated luciferase activity is significantly reduced compared with empty vector transfected control cells. Moreover, FACS scan analysis demonstrates that SFRP1 expressing MDA-MB-231 cells are more susceptible to death. We are presently utilizing a SFRP1 knockout mouse model to elucidate the involvement of SFRP1 in mammary gland development and breast cancer susceptibility. Taken together, these data could lay the foundation for the development of preventative treatments aimed at antagonizing the Wnt/β-catenin signaling pathway in patients with reduced levels of SFRP1.

Title:

Elucidating the Role of Wnt Signaling in Breast Cancer

Authors:

Theresa DiMeo, Kristen Anderson, Stephen Naber, Charlotte Kuperwasser

Presented by:

Theresa DiMeo

Department:

Department of Anatomy and Cellular Biology, Tufts University School of Medicine

Abstract:

The Wnt family of secreted proteins is essential for normal embryonic development, as well as self renewal and differentiation of adult tissues. Mutations in the Wnt signaling pathway (for example, APC) are well documented in promoting the initiation of colon cancer. Interestingly, mutations in the Wnt pathway have not been linked to the progression of breast cancer; however, many breast cancers exhibit aberrant stabilization of β-catenin and overexpression of Wnt ligands. Additionally, many negative regulators of the Wnt pathway are silenced in breast cancer.

Recently, we have shown that human breast cancer cell lines maintain tumor cell heterogeneity in culture (Fillmore et al 2008). A small subpopulation of cells in the cell line displays self-renewal properties to give rise to phenotypically diverse progeny and can initiate tumor growth *in vivo*. One such able cell line, the SUM1315 line, does not express endogenous sFRP1, a negative regulator of the Wnt pathway, and displays active Wnt signaling in culture. Given the importance of the Wnt signaling pathway in maintenance of self-renewal in various systems, we set out to elucidate the impact of inhibiting this pathway in breast cancer using a xenograft mouse model system. The secreted Wnt inhibitors sFRP1 and Dkk1 were transduced by retroviruses into the SUM1315 cell line and characterized for their phenotypes *in vitro* and *in vivo*. While both sFRP1 and Dkk1 act similarly to inhibit Wnt1-induced TOPFlash activity, serial CFU formation, and tumorsphere formation *in vitro*, only Dkk1 is able to inhibit primary tumor formation *in vivo*. Knockdown of Lrp6 in SUM1315 cells phenocopied the Dkk1-SUM1315 overexpressing cells, indicating that Dkk1 is acting through the canonical Wnt pathway to mediate its inhibitory effects. We found that both Dkk1 overexpression and shRNA mediated knockdown of Lrp6 promote differentiation of the SUM1315 cells to a myoepithelial phenotype. We are currently trying to elucidate the gene targets that are responsible for the reduction in tumor growth and are altered in response to Dkk1 expression.

Title:

Inhibitory Effects of *Rhodiola Crenulata* on an Invasive Immortal Human Cell Line

Authors:

Adaris Rodríguez-Cortés, Kelly Gauger, Sallie Smith Schneider

Presented by:

Adaris Rodríguez-Cortés

Department:

Pioneer Valley Life Sciences Institute, Baystate Medical Center

Abstract:

The root of the Tibetan plant *Rhodiola crenulata* is part of eastern traditional medicine. Studies have suggested that members of the *Rhodiola* genus display anticancer properties. In this study we examine the effect of *R. crenulata* in a cellular model of invasive breast cancer, this disease being the second cause of cancer death among women in the United States.

Deregulation of the Wnt/β-catenin pathway has been frequently observed in breast cancers and appears to have a key role in the transformation of benign cells to a malignant form. Although mutations of the Wnt growth factor are rarely observed in cancer, the Wnt signaling pathway is often up-regulated by either mutations that result in stabilization of β-catenin or by hypermethylation and subsequent loss of expression of Wnt signaling antagonists like secreted Frizzled-Related Protein 1 (SFRP1). We used an engineered cell line in which SFRP1 expression has been knocked down. These cells were derived from 76NTert cell line, an immortalized human mammary epithelium cell line. The resulting 76NTert-siSFRP1 cells display a mesenchymal-like phenotype, invasive behavior and are more resistant to apoptosis triggered by anchorage independent conditions, or anoikis. Treatment of 76NTert-siSFRP1 cells with an extract of *R. crenulata* inhibited migration and invasion of the 76NTert-siSFRP1 cells, as compared to untreated cells. Furthermore, *R. crenulata* sensitizes cells to anoikis but does not increase γ-irradiation induced cell death. We provide evidence that death induced by *R. crenulata* does not occur through the inhibition of an epithelial-to-mesenchymal transition (EMT). Taken together, our initial results suggest *R. crenulata* as a potential therapeutic agent for breast cancer patients with mutations in the Wnt/β-catenin signaling pathway.

Title:

Tumor-Targeted Delivery of TRAIL Using *Salmonella Typhimurium* Enhances Breast Cancer Survival

Authors:

Sabha Ganai, Richard Arenas, Neil Forbes

Presented by:

Sabha Ganai

Department:

Surgical Oncology Service, Baystate Medical Center

Abstract:

Background: Attenuated *Salmonella typhimurium* has been demonstrated experimentally as a novel anticancer agent because of its favored growth within tumors, limited toxicity, and antibiotic susceptibility. In order to study the ability of *S. typhimurium* to provide spatiotemporal control of cytotoxic protein delivery, a radiation-inducible expression system for secretion of TNF-related apoptosis-inducing ligand (TRAIL) was developed.

Methods: Prokaryotic-expression plasmids for TRAIL or green-fluorescent protein using the *RecA* promoter were electroporated into the *msbB*$^{-}$ *purI*$^{-}$ strain, VNP20009. In a syngeneic model of mammary carcinoma using BALB/c mice, the effect of systemic infection of bacterial vectors with or without induction by 2Gy gamma-irradiation at two days after colonization was assessed, examining outcomes of tumor growth and thirty-day survival.

Results: *In vitro* confirmation of extracellular TRAIL secretion and caspase-3 and caspase-8 activity were verified, with increased apoptosis measured by annexin-V/propidium iodide flow cytometry ($p<0.05$). The expression vector for TRAIL induced by radiation led to a significant delay in tumor growth and improved thirty-day survival *in vivo*, with a hazard ratio of 0.24 (95% confidence interval, 0.08–0.75; $p<0.05$) in comparison with irradiated controls. Repeated dosing and irradiation after one week limited tumor growth from baseline, with a significant survival benefit from 0% to 100% at one month after initial treatment ($p<0.05$).

Conclusions: By capitalizing on the intrinsic motility of bacteria and their preferential accumulation within tumors, the pre-clinical utility of targeted therapy using *Salmonella typhimurium* as a TRAIL expression vector has been demonstrated as an effective method to reduce tumor growth and improve host survival.

Title:

Disease Modeling and Tissue Engineering: Breast Cancer Metastasis to Bone

Authors:

Michaela Reagan, Robert Goldstein, Michael Rosenblatt, David Kaplan

Presented by:

Michaela Reagan

Departments:

Department of Biomedical Engineering, Tufts University School of Engineering; Department of Physiology, Tufts University School of Medicine

Abstract:

Osteotropism is a complex disease involving multiple causes and courses, with invasion and metastasis comprising 90% of cancer deaths. According to US statistics, breast cancer, one of the most common cancers to metastasize to bone, is the most frequently diagnosed cancer in women and the second most fatal, mainly due to metastasis. Understanding the underlying biological reasons for skeletal breast cancer metastasis is imperative for treatment and prevention. Our lab utilizes human tissue engineered (TE) bone, specifically designed with cellular, mineral, and growth factor components, in a disease model with NOD/SCID mice. Our TE bone is formed using porous, biocompatible, 3D silk fibroin scaffolds and human mesenchymal stem cells differentiated into osteoblasts. This TE bone has proven to be valuable in modeling metastasis to bone *in vivo* using the breast cancer cell line SUM1315. The model established species-specific metastasis from an orthotopic location to human TE bone, exclusively, and not to the mouse skeleton. TE bone is well defined physically, chemically, and biologically, and can also be analyzed with immunohistochemistry and RNA-extraction without decalcification. Building on previous findings, current studies are focused on expanding the *in vivo* disease model from bone tissue to bone marrow by using undifferentiated bone marrow-derived stem cells to establish a humanized *in vivo* model of metastasis to human bone marrow. We also hypothesize that different maturational stages of bone development cause different metastatic potentials. Hence, further studies are focused on characterizing TE bone development *in vitro* and *in vivo* over time to determine effects of bone maturation and bone components such as BMP2 on metastasis.

Title:

A Humanized Model of Breast Cancer Metastasis Revealing a Human-Specific Metastasis Gene Signature

Authors:

Robert Goldstein, Kristen Anderson, Michael Rosenblatt

Presented by:

Ellen Scepansky

Department:

Department of Physiology, Tufts University School of Medicine

Abstract:

The skeletal complications of malignancy represent some of the most serious complications associated with cancer, signaling the entry of the disease into an incurable phase. Despite many recent advances in cancer research and therapy, there remains a need for a better understanding of the metastatic spread of cancer from its primary location to distant "soils" within the body. Animal models are critical to the understanding of the complexities of tumor metastasis. Through creation of an animal model that is more representative of human pathophysiology, it should be possible to elucidate the genetic alterations that are required for cancer migration from orthotopic locations to distant sites. Further, clinical observations indicate that organ and stromal environments greatly influence the response of tumors to chemotherapy, suggesting that orthotopic implantation of tumor cells in animal models can recapitulate the process of cancer metastasis to bone. Currently, orthotopic implantation of cancer cells is not widely used in animal modeling. Work in our lab has developed a humanized orthotopic injection model of breast cancer metastasis. We use SUM1315 breast cancer cells injected into the murine mammary fat pad. Subsequently, metastases to subcutaneously implanted human bone cores develop. When compared to results obtained from an intracardiac injection model of cancer metastasis to murine bone, results from our gene array studies and genetic profiling have illuminated a novel metastatic gene signature. While different in specific gene identities (intracardiac signature=MMP1, IL-11, CTGF, and CXCR4; orthotopic signature=MMP13, IL-17BR, and HUNK), the two signatures contain genes of overlapping function (i.e., homing, invasion, angiogenesis and osteolysis). Comparison of the expression levels of the specific gene signatures across different breast cancer cell lines used in the intracardiac or the orthotopic injection models of breast cancer metastasis using qRT-PCR, provides the basis for our hypothesis, suggesting that the humanized animal model of breast cancer metastasis provides a novel, human-species specific, metastatic gene signature.

Title:

Role of Bone Sialoprotein (BSP) Overexpression in Osteolytic Metastasis in CMV-BSP Transgenic Mice

Authors:

Qisheng Tu, Jake (Jinkun) Chen, Jin Jang, Min Kim

Presented by:

Min Kim

Department:

Department of General Dentistry, Tufts University School of Dental Medicine

Abstract:

Objective: BSP is a protein normally found only in mineralizing tissues and is a major non-collagenous protein in bone. More recent studies indicate a positive correlation between the expression level of BSP and metastasis of tumor cells to bone. BSP is also a marker for tumor size, lymph-node status, and a poor prognosis for breast cancer patients. The objective of this study was to determine the role of bone sialoprotein (BSP) overexpression in osteolytic metastasis in our CMV-BSP transgenic mice.

Methods: We first generated transgenic mice, in which a mouse BSP is linked to a CMV promoter (CMV-BSP). 4T1 breast cancer cells were cultured and were transplanted into CMV-BSP transgenic mice as well as wild type littermates by intracardiac injection. At week 1, 2, 3 and 4 after the first set of injections, the tumor metastasis level and the bone resorption level were analyzed. As the transplanted 4T1 breast cancer cells express the luciferase gene, the transplanted cancer cells were easily detected and localized in the metastatic sites by an optical (IVIS) imaging system which tracks the luciferase expressing cells. X-ray analysis was used to measure the level of bone resorption. Four weeks after cancer cells transplantation, recipient animals were sacrificed. Half of the bone tissues with cancer metastases were used for histological analysis and the remaining half for RNA extraction. RT-PCR was preformed to determine the BSP and Luciferase expression levels in both experimental and control mice.

Results: The IVIS Imaging System demonstrated that among the mice receiving intracardiac inoculation, all the ten CMV-BSP mice developed metastases one week after injection while only four out of seven wild type mice showed metastatic lesions. The results demonstrated that BSP overexpression in the whole body and a high serum BSP level dramatically increase skeletal as well as systemic metastases of 4T1 murine breast cancer cells which originally show a primary characteristic of bone-seeking metastases. Autopsy, gross examination and histological analyses demonstrated that the CMV-BSP mice receiving 4T1 cell inoculation died soon due to multiple metastatic lesions involving vital organs such as liver, lung, and kidney before more osteolytic lesions were developed and could be detected.

Conclusions: 4T1 murine breast cancer cells caused more osteolytic metastasis in CMV-BSP transgenic mice than in wild type mice.

Title:

Targeted Overexpression of BSP in Osteoclasts Promotes Bone Metastasis of Breast Cancer

Authors:

Qisheng Tu, Amanda Fix, Jean Tang, Jin Zhang, Jake (Jinkun) Chen

Presented by:

Qisheng Tu

Department:

Department of General Dentistry, Tufts Univeristy School of Dental Medicine

Abstract:

Bone is one of the most common sites of breast cancer metastasis. Recent studies have shown that almost 90% of human breast tumors produce bone sialoprotein (BSP), a protein normally found only in mineralized tissues. We have recently found that BSP and receptor activator of nuclear factor kB ligand (RANKL) synergistically induce osteoclastogenesis and bone resorption and decrease apoptosis of osteoclasts. While BSP is thought to play an important role in bone metastasis of malignant tumors, there is no evidence confirming that BSP is directly responsible for osteolytic metastases.

The objective of this study is to determine the role of BSP overexpression in osteolytic metastasis *in vivo* by using a homozygous transgenic mouse line that constitutively overexpressed mouse BSP cDNA driven by an osteoclast specific cathepsin K (CtpsK) promoter.

We first generated the BSP overexpressing transgenic mice in which the BSP gene is driven by a promoter of osteoclast specific gene cathepsin K. Southern hybridization and PCR confirmed the integration of CtpsK/BSP chimeric gene into the mouse genome. An elevated level of expression of BSP in bone tissue and osteoclast cells was detected. RANKL induced osteoclast differentiation from bone marrow-derived monocytes/ macrophages and bone resorption were significantly greater in CtpsK/BSP transgenic mice than wild-type mice ($P<0.05$). Compared to wild-type mice, 6-week-old CtpsK/BSP mice had reduced trabecular bone volume (BV/TV%), cortical thickness (Ct.Th), and bone mineral density (BMD). Using an *in vivo* bone metastasis model and the real-time IVIS Imaging System, we found that 9 of 10 CtpsK/BSP mice that were injected with 4T1 murine breast cancer cells ($1x10^5$, labeled by luciferase reporter) developed large osteolytic bone lesions 3 weeks after injection. In contrast, only 4 of 9 wild type mice had lesions in their hindlimbs. The lesion area was significantly larger in CtpsK/BSP mice than in the controls as determined by X-ray, gross and histological analyses. It was found that targeted BSP overexpression in osteoclasts could activate the master regulator of osteoclastogenesis nuclear factor of activated T cells (NFAT)-2 and increased the mRNA expression of other differentiation markers such as cathepsin K or TRACP. We conclude that BSP is a mediator of osteolytic metastasis of malignant tumors through the induction of osteoclast activity, which provides the molecular basis of and treatment for bone destruction associated with osteolytic metastasis.

Title:

Treating Ductal Carcinoma in Situ (DCIS) of the Breast with Tamoxifen: A Decision Analysis of the Risks and Benefits of Warfarin Anticoagulation in a Patient at Increased Risk for Thromboembolism

Authors:

Stewart Evans, John Wong, Stephen Pauker

Presented by:

Stewart Evans

Department:

Division of Clinical Decision Making, Informatics and Telemedicine, Tufts Medical Center

Abstract:

Aim: We performed a decision analysis to determine if a 57-year old female with a history of atrial fibrillation, but without prior systemic emboli and DCIS, should receive lifelong anticoagulation and/or tamoxifen.

Methods: Using published data, we built a Markov model comparing four treatment strategies: combination therapy (warfarin plus tamoxifen); warfarin alone; tamoxifen alone; and neither warfarin nor tamoxifen. We considered risks of morbidity and mortality from major thromboembolism, major bleeding, and recurrent breast cancer that might occur over a lifetime (lifetime time horizon). To account for morbidity, we applied published quality of life measures.

Results: In the baseline analysis, when life expectancy alone was considered, treatment with warfarin alone yielded 21.7 years, compared with 21.5 years with neither warfarin nor tamoxifen, 21.3 years with combination therapy, and 19.6 years with tamoxifen alone. When quality of life was also considered, combination therapy yielded 19.7 quality-adjusted life years (QALYs), compared with 19.6 QALYs with warfarin alone, 19.5 QALYs with no medication, and 18.3 QALYs with tamoxifen alone. All parameters were varied over plausible ranges in sensitivity analyses. Combination therapy remained the preferred strategy over warfarin alone (other strategies were inferior) unless: the annual probability of thromboembolism without tamoxifen was higher than 1.1% (baseline 1.0%), the annual probability of breast cancer recurrence was below 2.6% (baseline 3.0%), the efficacy of warfarin was below 0.61 (baseline 0.65), the quality of life taking tamoxifen was below 0.99 (baseline 1.00), or the efficacy of tamoxifen was below 0.29 (baseline 0.32). Neither warfarin nor tamoxifen became preferred over all other strategies if quality of life while taking warfarin fell below 0.98 (baseline 0.99).

Conclusions: This analysis was consistent with current guidelines for breast cancer risk reduction with tamoxifen and reflects the complexity of the decision, particularly for this postmenopausal patient with an increased risk of thromboembolism secondary to atrial fibrillation. Tamoxifen alone was clearly the least preferred strategy; the choice among the other strategies was a relatively close call. Our analysis demonstrates how patient preferences and carefully individualized calculation of risks and benefits might inform the decision-making process.

Title:

Determining the Role of Immune System Function in Breast Cancer Using an Imagable Syngeneic Model of Breast Cancer

Authors:

Min Fang, Kai Tao, G. Gary Sahagian

Presented by:

Min Fang

Departments:

Department of Physiology, Tufts University School of Medicine; Molecular Oncology Research Institute, Tufts Medical Center

Abstract:

The 4T1 mouse mammary tumor model is one of only a handful of models that metastasize effectively when introduced orthotopically in immuno-competent mice. Because of its propensity to metastasize to bone and other sites in immuno-competent animals, the model is being used extensively for identifying drug targets and testing therapeutics aimed at inhibiting processes related to tumor metastasis. For this study, the 4T1 cell line was modified for stable expression of firefly luciferase in the absence of selective pressure and for targeted integration of siRNAs and cDNAs with FLP recombinase. Luciferase expression allows quantitative analysis of primary tumor growth and facilitates the detection and quantitation of metastases to lungs, liver and bone, as well as to previously unreported organs including brain, kidney and adrenals. A longitudinal experiment following mammary tumor growth and metastasis weekly after introduction of tumor cells into syngeneic BALB/c mice, revealed biphasic growth at the primary site with rapid growth during the first two weeks, regression in weeks 3 and 4, and renewed growth in weeks 5 and 6. A progressive increase in extramedullary hematopoiesis in spleen and liver occurred during the 6-week period. Regression in weeks 3 and 4 was associated with extensive inflammation and necrosis at the primary site. Increasing levels of neutrophils, monocytes and lymphocytes were observed in the circulation during the experiment and many of these cell types were recruited to the primary site. Growth at the primary site was substantially affected by the immune system function, in that regression of growth observed in normal mice was not seen in immuno-compromised BALB/c nude or SCID mice.

Metastases appeared during the second growth phase suggesting the involvement of innate and acquired immune responses in metastasis for this model. Microarray analysis comparing the gene expression in the 4T1 cell line to 67NR and 168FARN, two non-metastatic sibling cell lines, was carried out to investigate the molecular basis for the 4T1 metastatic phenotype. Pathway analysis of genes with significant expression differences indicated activation of the p38 MAPK signaling resulting in production of hematopoietic factors Csf2 and Csf3, acute

phase proteins Ssa3 and C3, metalloproteinases Mmp3, Mmp9 and Mmp13 and cytokines Ccl5, Cxcl16, Cxcl1 and Tslp. Factors involved in angiogenesis, including Vegfc and Agpt2, were also significantly over-expressed in 4T1 cells. Further analysis of the interactions of these genes, and the pathways and processes in which they function, suggest that factors produced by this breast cancer model orchestrate the production and recruitment of cells of the innate and acquired immune responses, which in turn regulate tumor growth and modify the intratumoral environment to allow dissemination of tumor cells to distant sites. The results highlight the importance of innate and acquired immune system function in tumor growth and metastasis, and emphasize the need for using immuno-competent animals and species matched models for the study of tumor metastasis.

Title:

Molecular Basis for Intravasation and Metastasis to Lung Using an Orthotopic Mouse Model of Breast Cancer

Authors:

Min Fang, Kai Tao, G. Gary Sahagian

Presented by:

Min Fang

Departments:

Department of Physiology, Tufts University School of Medicine; Molecular Oncology Research Institute, Tufts Medical Center

Abstract:

As an approach for identifying genes involved in intravasation and metastasis to lungs, variant luciferase-expressing 4T1 tumor cells were isolated from lung metastases after orthotopic injection of the parental line into the mammary gland of BALB/c mice. Pools of tumor cells isolated after one and two rounds of injection and metastasis (4T1-L1 and 4T1-L1L1, respectively) and two cell lines cloned from the 4T1-L1 pool (4T1-L1-c7b and 4T1-L1-c12b) were shown to metastasize more effectively to lungs after injection into the mammary gland. Analysis of cell doubling times in normal and immuno-compromised BALB/c mice, intravasation *in vivo*, and migration *in vitro*, indicated that the selected cells had increased ability to escape from the primary tumor and evade immune system clearance mechanisms. Comparison of gene expression profiles for selected cell pools and lines to the parental line revealed loss of tight junction components, increased expression of extracellular proteases, and other alterations associated with EMT (epithelial-mesenchymal transition). Expression of Sip1/ZEB2, a member of the δEF-1 family of two-handed zinc finger nuclear factors and a known effector of EMT, was elevated in the selected cells suggesting a role for this transcription factor in the metastatic phenotypes that were observed. Silencing and overexpression experiments and analysis of expression of Sip1/ZEB2 in human breast tumors indicated Sip1/ZEB2 plays an important role in the induction of EMT and the establishment of lung metastases in breast cancer.

Title:

Use of a Standardized Web-Based Tool for Evaluation of Bone Health in Breast Cancer Patients

Authors:

Konstantinos Arnaoutakis, Glenn Wong, Rekha Parameswaran

Presented by:

Konstantinos Arnaoutakis

Department:

Division of Hematology-Oncology, Caritas St. Elizabeth's Medical Center

Abstract:

Objectives: ASCO guidelines recommend aromatase inhibitors (AI) adjuvant therapy in post-menopausal hormone receptor positive breast cancer patients. Aromatase inhibitors can cause significant bone loss over the long-term and increase treatment related morbidity. This study measures a web-based tool use for osteoporosis evaluation, using the American Board of Internal Medicine Practice Improvement Module (ABIM PIM) for osteoporosis.

Methods: We performed a retrospective chart review (2004-06) of Breast Cancer Patients (BCP) on adjuvant AI without prior tamoxifen use; documentation of osteoporosis risk factors and processes of care were reviewed using the ABOIM PIM for osteoporosis.

Results: Of 228 BCP identified from the hospital tumor registry, 28 (93% Caucasian, 7% race not documented) were eligible for analysis; mean age was 64 years (range 42-90). Post-menopausal status was documented by history in 26 (92%); by LH, FSH and estradiol levels in 2 (8%) BCP. All tumors expressed estrogen receptors; 26 (92%) expressed progesterone receptors. Chart review revealed documentation of tobacco use in 19 (71%); exercise level in 12 (43%); fall screen in 1 (4%); vitamin D level in 0 (0%); appropriate vitamin D intake in 10 (36%) and calcium intake in 6 (21%) charts respectively. Bone density scan was done in 21 (75%) BCP. Of 17 (60%) BCP eligible for antiresorptive therapy; only 1 (6%) had documentation of prescribed therapy.

Conclusions: The ABIM PIM osteoporosis survey is a useful web-based tool in AI treated BCP. It identifies patients at high risk of osteoporosis and reveals deficiencies in chart documentation. By identifying high-risk patients in a timely fashion, management modifications can be made, potentially reducing osteoporosis related complications. We conclude that use of this web-based tool in select patient populations enhances quality of patient care and could potentially decrease treatment related morbidity.

Title:

Progenitor-Progeny Relationships of the Marker Defined Cell Subsets in the Human Prostate

Authors:

Andrew Makarovskiy, Peter Geck, Allen Parmelee, Albert Tai, Gennaro Carpinito

Presented by:

Andrew Makarovskiy

Departments:

Departments of Anatomy and Cellular Biology and of Pathology, Tufts University School of Medicine;
Department of Urology, Tufts Medical Center

Abstract:

There is a gap in understanding stem cell lineages in the prostate and our knowledge is also incomplete about how stem cells become involved into malignant prostate tissue growth. Consequently, this limits our ability to develop new therapy alternatives to target and eliminate prostate tumor initiating cells. A recent landmark study showed that CD133+ prostate tumor cells can be clonogenic when injected into male mice. We used a panel of new monoclonal antibodies (MAb) against differentially displayed rare surface antigens to further characterize the stem cell populations of the prostate. The new MAbs define subsets of prostate epithelial cells both in fetal and adult normal prostate, which persist in prostate carcinomas. MAb specificity to the cell subsets was also retained *in vitro*. One of the new antibodies (SCS7) consistently labeled a rare population of cells residing in the stroma of the developing and adult prostate. Analysis showed their immature morphology and phenotype and indicated lack of proliferative activity. Sporadic intermediate phenotypes detected by multicolor fluorescent microscopy using the new and conventional cell type specific markers provided antigenic links to the prostate parenchyma and suggested SCS7+ cells are not a stromal cell subset. Importantly, quiescent in normal tissues, SCS7+ cells, demonstrated mitotic activity following androgen deprivation, suggesting androgens might be acting as a negative regulator of SCS7 proliferative activity and withdrawal of androgens removes the proliferative blockade. A similar phenomenon was observed *in vitro*, following purification of the normal adult SCS7+ cells which were able to produce a progeny with phenotype of the prostate basal cells, suggesting a higher lineage position of SCS7+ cells. To establish a germane link between the CD133+ prostatic stem cells and the SCS7+ population, we analyzed these cell subsets, using both normal and PC-3 carcinoma cells, by FACS. Analysis indicated that either population was present at a <1% rate with a minor overlap suggestive of transitional phenotype. To determine their progenitor-progeny relationship, we purified these subsets from prostate carcinoma cell line PC-3 first. While the morphology and growth characteristics of the fractionated subsets were similar, the progeny differed in phenotype. SCS7+/CD133- cells produced SCS7+/CD133-, SCS7+/CD133+ and SCS7-/CD133+ subsets as in parental cultures. On the contrary, the CD133+/SCS7- subset was unable to generate SCS7+ progeny suggesting restricted differentiation potential of the CD133+ stem cells. We hypothesize that a network of resident stem cells that includes phenotypically and functionally different stem cell types maintains the human prostate lineage. Our future studies will compare the differentiation potential and androgen responsiveness of these cell subsets *in vivo*.

Title:

New Target Genes in Prostate and Breast Cancer: Methylation and MicroRNA Silencing of a Stem Cell Differentiation Gene, APRIN, in Clinical Studies

Authors:

Monika Pilichowska, Viktoria Denes, Byung Kyu Kim, Andrew Makarovskiy, Gennaro Carpinito, Peter Geck

Presented by:

Peter Geck

Departments:

Departments of Pathology and of Urology, Tufts Medical Center; Department of Anatomy and Cellular Biology, Tufts University School of Medicine

Abstract:

Focus Area: New targets

Introduction: Differentiation programs are disrupted early in prostate cancer. Disruptions of differentiation genes, therefore, may be the earliest diagnostic markers of cancer. In our search for new target genes in cancer, we searched for novel genes in differentiation that also play a role in cancer. We isolated a nuclear protein, APRIN, and we found that APRIN was involved in stem cell differentiation. APRIN mutations in the germ line were shown to generate extensive birth defects (Cornelia de Lange Syndrome), suggesting a critical regulatory function for APRIN. We found that the APRIN gene was silenced in a variety of cancers, e.g., it was downregulated or silenced in 70-80% of breast and ovarian carcinomas.

Objectives: The goals of this project were to investigate:

(i) if APRIN was also silenced in prostate cancer;

(ii) to identify the mechanisms involved; and

(iii) to investigate the correlation between APRIN silencing and cancer, as an early marker for prostate cancer diagnosis or prognosis.

Methods: Clinical cancer samples were investigated for APRIN silencing at three levels:

(i) Protein expression: We used polyclonal anti-APRIN antibodies on formalin fixed, paraffin embedded cancer samples (immunohistochemistry).

(ii) Promoter methylation analyses: Microdissected DNA samples were bisulfite converted. We used methylation sequencing and methylation-specific quantitative PCR.

(iii) MicroRNA-based mechanisms: Total RNAs were prepared from microdissected paraffin sections (Trizol). MicroRNA levels were detected by quantitative miRNA PCR.

Results:

(i) Immunohistochemistry showed high APRIN levels in normal prostatic epithelial cells. In prostate cancer, we found APRIN silencing in 30-50% of the samples.

(ii) Promoter methylation studies detected methylation hot-spots in the APRIN promoter. Methylation assays by Q-PCR indicated 20-80% methylation levels in the prostate carcinoma samples we studied.

(iii) APRIN-specific microRNAs were predicted by computer analysis (PicTar, MiRNA-Viewer, TargetScan, MiRBase etc.). We identified hsa-miR-17-3p, 27a, 200 and 223 that may potentially target APRIN. In cell lines where APRIN was silenced by miRNA-based mechanisms, miR-27a and 200 were highly increased. In the control lines where APRIN was highly expressed, microRNA levels were low. In prostate cancer samples, our pilot studies with miRNA Q-PCR showed that in cancers where APRIN was silenced, miR-17-3p, 27a and 200 were highly upregulated.

Conclusions: APRIN regulates differentiation programs in reproductive and other tissues. We showed that oncogenesis in the prostate involves APRIN silencing through promoter methylation and microRNA mechanisms. Our ultimate goal is to establish novel APRIN epigenetic markers for early diagnosis and prognosis.

Title:

β1 Integrin Participates in Endoglin-Dependent Inhibition of Prostate Cancer Cell Migration

Authors:

Diana Romero, Jennifer Roth, Peter Brooks, Calvin Vary

Presented by:

Diana Romero and Calvin Vary

Department:

Center for Molecular Medicine, Maine Medical Center Research Institute

Abstract:

Endoglin is a transmembrane glycoprotein involved in the regulation of TGF-β signaling. PC3-M metastatic prostate cancer cells have undetectable levels of endoglin, whereas their non-metastatic counterpart expresses endoglin. When the expression of endoglin was restored in PC3-M cells, we observed a (cytoplasmic domain) CD-dependent inhibition of cell migration and a reduction of the tumorigenicity of PC3-M cells in scid mice. Using these cells, we determined that endoglin phosphorylation by the TGF-β receptors ALK2 and ALK5 is a mechanism involved in TGF-β1-dependent regulation of cell migration.

Our current objective is to analyze the signaling pathways downstream of endoglin that lead to the inhibition of prostate cancer cell migration and invasion. We have observed that endoglin expression has a dramatic effect in the organization of the actin cytoskeleton in PC3-M cells. Interestingly, endoglin co-localized with the actin fibers in focal adhesion sites. In addition, endoglin expression affected PC3-M cell adhesion on the extracellular matrix proteins collagen types I and IV, suggesting that there is a functional proadhesive interaction between β1 integrin and endoglin. Further, elucidation of these interactions will provide a mechanistic explanation for endoglin regulation of cell migration in a highly metastatic prostate cancer cell line.

Title:

PVR/Necl-5 Promotes Glioblastoma Invasion by Activation of the Akt Pathway and MMP-2 Production

Authors:

Brian Enloe and Daniel Jay

Presented by:

Brian Enloe

Department:

Department of Cellular and Molecular Physiology, Tufts University School of Medicine

Abstract:

Glioblastoma is a severe brain cancer, with medial survival of approximately one year after diagnosis, even when treated aggressively with surgery, chemotherapy, and radiation. Treatment failure is due to tumor recurrence, which is the result of the cancer cells ability to invade from the main tumor into the surrounding areas of the brain. PVR/Necl-5 was identified by our lab as a protein involved in glioblastoma cell invasion using a functional proteomic screen. PVR/Necl-5 is a transmembrane protein that is localized at the leading edge of invading cells. Depletion of PVR/Necl-5 using RNAi leads to decreased invasion, and reduced secretion of matrix metalloproteinase 2 (MMP-2). Glioblastoma severity *in vivo* correlates with MMP-2 expression, and this protease is responsible for degrading the extracellular matrix. PVR/Necl-5 depletion also results in reduction in activity of integrin-linked kinase (ILK) as measured by reduced Akt phosphorylation at serine 473. Depletion of ILK leads to a nearly complete loss of MMP-2 production. Thus, we propose that PVR/Necl-5 links ILK and Akt activation, resulting in increased expression of MMP-2.

Title:

Role of Histone H3K27 Demethylases in Maintenance of Multipotency in Glioblastoma Stem Cells

Authors:

Maureen Sherry, Andrew Reeves, Julian Wu, Brent Cochran

Presented by:

Maureen Sherry

Departments:

Department of Physiology, Tufts University School of Medicine; Department of Neurosurgery, Tufts Medical Center

Abstract:

Signal transducer and activator of transcription-3 (STAT3) is an important mediator of growth factor and cytokine responses. It is constitutively activated in a variety of human cancers. In addition, STAT3 is indispensable for murine embryonic stem cell self-renewal, neural stem cell self-renewal, and astrocyte differentiation. In order to determine whether STAT3 has a similar role in glioblastoma stem cells, we have isolated several stem cell lines from primary human GBM tumor samples. We have found that inhibition of STAT3 with small molecules as well as with RNAi causes growth inhibition in the GBM stem cells. Markers of multipotency such as Olig2 and nestin are also decreased upon STAT3 inhibition. Strikingly, STAT3 inhibition is irreversible. Cells exposed to the STAT3 small molecule inhibitor STA-21 for as little as 4 hours do not regain the ability to form neurospheres while still remaining viable. This observation suggests that STAT3 may regulate epigenetic changes in glioblastoma. It is clear that the multipotency of stem cells is maintained in part by the polycomb repressor complex, which inactivates expression of differentiation-specific genes by methylation of histone H3 on lysine 27. Histone modifying enzymes have recently been found that demethylate this residue and lead to derepression of the polycomb regulated genes. One of these demethylases, Jmjd3, appears to mediate neuronal differentiation in mice. We have found that inhibition of STAT3 leads to a 100 fold increase in expression of the Jmjd3 demethylase mRNA. This induction is rapid (within 1 hour), suggesting that Jmjd3 is directly regulated by STAT3. This induction is accompanied by an increase in the expression of known Jmjd3 target genes, providing further evidence that Jmjd3 may be mediating epigenetic changes in glioblastoma stem cells. The ability of STAT3 to modulate epigenetic changes via its regulation of Jmjd3 may explain the irreversibility of STAT3 inhibition in GBM-SC. Thus, targeting STAT3 may provide an effective and potentially irreversible means of depleting the cancer stem cell population in glioblastoma.

Title:

A Conditional RNAi Strategy to Investigate Signaling Networks in Glioblastoma Multiforme

Authors:

Jaime Acquaviva, Pranatartiharan Ramachandran, Steve Woolfenden, Al Charest

Presented by:

Jaime Acquaviva

Departments:

Department of Neurosurgery and Molecular Oncology Research Institute, Tufts Medical Center

Abstract:

Glioblastoma multiforme (GBM) is the most common and aggressive form of malignant astrocytic glioma. These rapidly fatal intracranial tumors are characterized by a high rate of cell proliferation, diffuse infiltration, and resistance to traditional and targeted therapy. Increased receptor tyrosine kinase (RTK) activity and subsequent activation of various downstream signaling pathways is often associated with GBM and thought to contribute to tumor initiation and maintenance. Here, we investigate the potential of an RNAi based system to study important GBM signaling events. Amplification and/or mutation of the epidermal growth factor receptor (EGFR) gene are common genetic alterations in GBM. An EGFR mutant allele with deletion of exons 2-7 (known as EGFRvIII) results in expression of a constitutively active variant of EGFR and is the most frequent EGFR mutation found in GBM. The tumorigenic properties of EGFRvIII are well established making it an attractive therapeutic target. We show conditional, lentiviral driven, shRNA mediated knockdown of EGFRvIII in the human GBM cell line, GBM6. To apply this strategy *in vivo*, we modified a population of lentiviral-shRNA infected GBM6 cells to express luciferase and injected them into the striatum of nude mice. Our results show that intracranial tumors can be established using human GBM cells modified for conditional RNAi and tumor progression can be monitored by *in vivo* bioluminescence imaging (BLI). In addition, conditional knockdown of EGFRvIII is retained in primary tumor derived cultures. We are currently using this orthotopic xenograft model to optimize conditions for effective shRNA mediated knockdown of EGFRvIII *in vivo*. Achieving sustained EGFRvIII knockdown *in vivo* will lead to valuable insight regarding the role of EGFRvIII in GBM and, importantly, validate the use of this technique to study other signaling components relevant to GBM. This GBM model system will provide a means to assess the potential of RNAi based therapeutics *in vivo* and enable us to establish functional links between signaling pathways and various aspects of GBM tumor biology.

Title:

Somatostatin Therapy for Recurrent Meningiomas

Authors:

Abdullah Kandil, Jay Zhu, Carl Heilman, Julian Wu

Presented by:

Abdullah Kandil

Department:

Boston Institute of Neurosurgery, Tufts Medical Center

Abstract:

Introduction: Most meningiomas have been shown to overexpress somatostatin receptor 2, subtype 2A (sst2A) and somatostatin has been found to inhibit meningioma growth *in vitro*. Recently, Chamberlain et al. have demonstrated a response to Sandostatin, an extended release somatostatin analog, in patients with recurrent meningiomas.

Methods: By using the same protocol as reported by Chamberlain et al., we enrolled patients with recurrent, atypical or malignant meningiomas who had measurable tumors by neuro-imaging as well as by ^{111}In-octreotide SPECT scanning after previous surgeries and radiation therapies. Sandostatin was administered subcutaneously every month. Most patients were followed with monthly blood counts and neurological examinations. Responses were determined by contrast-enhancing neuro-imaging every 3 months. Toxicity is monitored and graded according to the Common Toxicity Criteria (CTC) version 2.0.

Results: Over a 10 month period, 3 males and 3 females with a median age of 70.5 years (range from 34-87 years) were enrolled. Median time of follow-up was 9.5 months (range from 5-10 months). One patient died 9 months later due to progressive disease. Radiographic evaluation showed no reduction of tumor sizes among all 6 patients. Three patients had grade I reaction with fatigue and injection site skin erythema. No grade 2 to 4 toxicity was observed.

Conclusion: In our small series of 6 patients with recurrent meningiomas with a median follow-up of 9.5 months while on somatostatin analog therapy, there was no radiographic response by the tumor that was detected in any patient. Although it is well tolerated, we conclude that somatostatin alone is not likely to halt meningioma progression.

Title:

Clinical Impact of Adjuvant Gamma Knife Radiosurgery to the Surgical Cavity of Resected Metastases: A Retrospective Review of the Tufts Medical Center Experience

Authors:

Steven Hwang, Rebecca Eisenberg, Andrew Hale, Tomas Dvorak, Abraham Boskovitz, Kevin Yao, Rolf Pfannl, John Mignano, Jay Zhu, Gary Strauss, Julian Wu

Presented by:

Julian Wu

Departments:

Departments of Medicine, of Neurosurgery, of Neurology, of Pathology, and of Radiation Oncology, Tufts Medical Center

Abstract:

Purpose: To help define the role of stereotactic radiosurgery (SRS) in treating brain metastases, we analyzed the impact of Gamma Knife (GK) stereotactic radiosurgery to the tumor cavity following surgical resection of brain metastases.

Patients and Methods: In this retrospective cohort study, 48 patients underwent surgical resection of at least one brain metastasis between December 1999 and December 2005. Thirty-two received post-operative GK treatment while the remaining did not. Both univariate and multivariate Cox proportional hazards regression were utilized to model the influence of various prognostic factors on survival.

Results: The median survival of the entire group was 18.3 months. Patients treated with GK achieved a median survival of 19.3 months as compared to 6.6 months for the untreated group ($p=0.43$). As expected, younger patients (<50 years) had longer mean survival than older patients (32.3 versus 9.2 months). However, GK did not appear to influence survival in the younger subset, but did have an impact on the older group (9.2 vs 18.6 months, $p=0.019$).

Conclusions: Our study suggests that the addition of GK to surgical resection of cerebral metastases may contribute to improved survival in patients. This report suggests that adjuvant SRS to the resection cavity may improve outcome in some of these patients. No decline in survival was seen in any subset, and the addition of GK to surgical resection appears to be particularly beneficial among patients >50 years of age. We conclude from our study that Gamma Knife radiosurgery to the surgical resection cavity may contribute to improved survival without significant additional morbidity in patients with brain metastases.

Poster No. 28A

Title:

Improvement of Neurological Function after Concurrent Treatment IVIG and Chemotherapy in a Patient with Cerebellar Degeneration Paraneoplastic Syndrome

Authors:

Jay-Jiguang Zhu, XueMei Li, Thomas Hedges, Katie Wakeley

Presented by:

Jay-Jiguang Zhu

Departments: Departments of Neurology, of Ophthalmology, and Divisions of Hematology and Oncology, and Gynecologic Oncology, Tufts Medical Center

Abstract:

Paraneoplastic syndrome is a rare manifestation of systemic cancers, often associated with lung or ovarian malignancy. Paraneoplastic cerebellar degeneration (PCD) is one of the common presentations of paraneoplastic syndrome. Clinically, patients present with cerebellar symptoms including dysarthria, gaze evoked nystagmus, as well as, gait ataxia. Frequently, anti-Purkinje cell antibodies, (anti-Yo) are detected in the serum and cerebrospinal fluid. There is no effective treatment for such conditions. Intravenous immunoglobulin (IVIG) is one of the commonly used therapies, but with limited success.

We reported a 55 year old woman with ovarian cancer, who was diagnosed with paraneoplastic cerebellar degeneration, with clinical manifestation of cerebellar syndrome and positive anti-Yo antibody (1:1500 dilution) in CSF only and mild elevation of tumor markers for ovarian cancer, CA-125. She presented with one and a half months history of sub-acute onset of dysarthria, gait ataxia and intermittent blurry vision with upright position. Exam showed downbeat nystagmus and alternating skew deviation, dysmetria, as well as gait ataxia. Incidentally, her symptoms significantly improved 1 week after a routine influenza vaccine injection. However, it did not last long and the same symptoms recurred 2-3 weeks later. Magnetic resonance imaging (MRI) head with and without contrast, at the time of diagnosis, did not reveal cerebellar atrophy. She received IVIG at 0.4 gm/kg/day x 5 days every 4 weeks with concurrent chemotherapy with intravenous doxorubicin (Doxil) and cyclophosphamide (Cytoxan) monthly. Three months after such treatment, her speech and dysmetria have markedly improved. She continued to receive the treatment monthly for a total of 12 cycles. Updated outcome of this patient and review of current treatment options of paraneoplastic syndromes will be reported at the meeting.

Title:

Larger Lentigo Maligna Lesions and Invasive Melanoma: Size Matters

Authors:

Priya Zeikus, Jyotsna Kakullavarrupu, Suzanne Olbricht

Presented by:

Suzanne Olbricht

Departments:

Departments of Dermatology and of Research, Lahey Clinic; Department of Dermatology, University of Texas Southwestern Medical Center

Abstract:

Background: Lentigo maligna is traditionally recognized as a slow growing lesion that undergoes malignant transformation to invasive melanoma. Treatment regimens for lentigo maligna vary widely from watchful waiting to surgical excision via Mohs technique as it can not be predicted which lentigo maligna lesions develop invasive melanoma.

Objective: 1) To examine the cases of lentigo maligna that were found to be invasive after surgery was performed, and 2) To determine whether specific clinical features exist that can predict invasive potential.

Methods and Materials: Eighty-nine cases of lentigo maligna that underwent staged excision were retrospectively reviewed. Clinical features and treatment details were recorded. Pathological reports were reviewed for the presence of invasion. Statistical analysis was performed with both Fisher T and Pearson Chi square tests.

Conclusion: There were 13 (14.6%) lesions that were found to have invasive melanoma only after subsequent slow Mohs excision. A strong correlation was observed between large lesion size and invasive potential. Nine of 18 (50%) of lesions greater than 4 cm^2 area were invasive compared to 4 of 71 (5.6%) less than 4 cm^2 ($p<0.0001$). There was no correlation of invasion based on gender, age, number of Mohs stages, length of time between biopsy and excision, or previous treatment rendered. Superficial therapies for larger lentigo maligna lesions are absolutely contraindicated and must be used with extreme care for smaller lesions which still have a significant albeit smaller risk for invasive melanoma.

Title:

Mapping Genes Associated with Spontaneous Canine Hemangiosarcoma and Expression Profiling, Potential Animal Model for Human Angiosarcoma

Authors:

Chieko Azuma, Kerstin Lindblad-Toh, Elinor Karlsson, Noriko Tonomura, Lisa Barber, Kristine Burgess, Scott Shaw, John Keating, Dawn Meola, Jun Xu

Presented by:

Chieko Azuma

Departments:

Departments of Clinical Sciences and of Biomedical Sciences, Cummings School of Veterinary Medicine

Abstract:

Spontaneous canine hemangiosarcoma (HSA), a malignant tumor of vascular endothelial cells, is a significant health concern in dogs. A particularly high disease incidence in Golden Retriever (15%) and certain other breeds suggests a genetic susceptibility and adds considerably to the power of mapping the genetic risk factor. HSA metastasizes rapidly through a hematogenous route or local seeding after tumor rupture. The median survival time with surgical removal of a splenic HSA with or without chemotherapy is 2-5 months. We have proposed to map genes associated with an increased risk of HSA in Golden Retrievers, as well as other, breeds in collaboration with the Broad Institute of Massachusetts Institute of Technology and Harvard University. Artificial forces for the breed creation in dogs provide a unique opportunity to study genetics for complex diseases and the application for use in human diseases. Characteristics of canine genetic structures include longer Linkage Disequilibrium (LD) and smaller variation of haplotypes within the breeds compared to general human population resulting in a higher chance to identify genes associated with complex diseases in dogs with smaller number of subjects.

Genome-wide association mapping with ~50,000 SNPs (Affymetrix canine SNP array) in 100 Golden Retrievers with HSA and 102 normal Golden Retrievers has identified 7 preliminary loci. Six loci have been validated by fine mapped in 5 breeds including Golden Retrievers, Labrador Retrievers, German Shepherds, Leonbergers and Boxers. All loci are found in at least one or more breeds and are discrete in size (<600kb). So far no coding mutations have been identified. We are also investigating their functional consequences by an expression profile of the canine HSA and to test if the mutations alter the expression of any of possible candidate genes and related pathways. Once the mutations have been identified and their presence in different breeds assessed, this will allow for rapid development of genetic tests for carriers of HSA. Furthermore, canine HSA resembles human angiosarcoma and may serve as an excellent model for this and other cancers with a high metastatic potential. Ultimately, understanding of the disease biology will lead to identify target genes for prevention, early detection and novel therapeutics in dogs and humans with cancer.

Title:

YKL-40 Demonstrates a Protective Role in Rhabdomyosarcoma by Decreasing MMP-2

Authors:

Steve Scully, Wie Yan, Rong Shao

Presented by:

Steve Scully

Department:

Pioneer Valley Life Sciences Institute, Baystate Medical Center

Abstract:

YKL-40 is a secreted glycoprotein, which has gained much attention as a sensitive and specific biomarker for several inflammatory diseases and as a prognosticator in thirteen different carcinomas. It is a chitinase like protein without chitinase activity and is phylogenetically conserved among animals, especially mammals. It is present and secreted in numerous cell types and tissues during development and is present in the most metabolically active cells in adult tissues under normal physiological conditions. Despite all of its recognition, little is known about the functions of YKL-40 in human development, normal physiology and disease. Some data have shown that YKL-40 plays a role in tissue remodeling. Since the case for YKL-40 as a biomarker has been made in multiple carcinomas, we decided to explore a similar role in sarcomas. We chose to look at Rhabdomyosarcoma since it is the most common soft tissue sarcoma in children and adolescents and results in high morbidity and mortality.

Our pilot data showed that YKL-40 was not expressed among various types of Rhabdomyosarcomas *in vitro*. Therefore, we decided to examine what role, if any, YKL-40 could play if it was overexpressed in these cancers *in vitro*. These studies demonstrated that YKL-40 decreased invasion, survival and proliferation. YKL-40 overexpression also reduced the levels of Matrix Metalloproteinase-2 and N-Cadherin in the RD cell line. Previous evidence has shown that Rhabdomyosarcomas are dependent on Matrix Metalloproteinases (MMPs) for their invasive and metastatic capabilities. In particular, MMP-2, MMP-9 and collagenase MMP-1 overexpression seem to contribute to the more aggressive phenotype of Rhabdomyosarcoma. Thus, further understanding of YKL-40 and its role as an inhibitor of MMPs in Rhabdomyosarcoma might lead to the development of novel anticancer therapies.

Title:

MicroRNA Expression Profiling: Connecting Lung Development and Lung Cancer

Authors:

Sana Mujahid, MaryAnn Volpe, Heber Nielsen

Presented by:

Sana Mujahid

Departments:

Department of Cell, Molecular and Developmental Biology, Tufts University School of Medicine;

Department of Pediatrics, Tufts Medical Center

Abstract:

MicroRNAs are a class of small RNAs that regulate gene expression by imperfectly pairing with the 3'UTR sequence of their target mRNAs, resulting in translational repression. Previous studies report a correlation between those genes up regulated during lung cancer and those involved in controlling normal fetal lung development. Our lab has been studying mechanisms necessary for normal fetal lung development. We recently have begun to study how miRNA mechanisms contribute to the regulated expression of genes involved in lung development. We have begun by using a miRNA qRT-PCR based screen of fetal mouse lungs during the critical period of development from a pseudoglandular to a saccular lung in preparation for survival at birth. One goal of this study is to find specific miRNA species which actively regulate gene expression for normal development and which also become reactivated during the progression of lung cancer.

Title:

Fibroblasts Affect the Phenotype of Normal Human Bronchial Epithelial Cells when Co-Cultured in Three-Dimensional (3D) Organotypic Cultures

Authors:

Steven Pageau, Maricel Maffini, Ana Soto, Carlos Sonnenschein

Presented by:

Steven Pageau

Department:

Department of Anatomy and Cell Biology, Tufts University School of Medicine

Abstract:

The stromal microenvironment plays an important role in the development and progression of adult respiratory diseases. Pulmonary diseases such as asthma, fibrosis, and cancer are thought to be the result of altered communications between epithelial and mesenchymal cells. In order to study epithelial and mesenchymal interactions *in vitro*, we have developed a three dimensional (3D) organotypic model of the human peripheral lung. This model consists of a type I collagen gel, normal human fetal lung fibroblasts (IMR-90), or primary human adult lung cancer-associated fibroblasts (LuCAFs), derived from lung cancer resective surgery and a surface epithelium of normal human bronchial epithelial cells (NHBECs). Our studies revealed that collagen gels lacking fibroblasts failed to promote the differentiation of a typical peripheral respiratory epithelium. Collagen gels containing NHBECs and IMR-90 fibroblasts at a ratio of 10:1 supported the development of an extensive surface respiratory epithelium containing goblet, basal, and ciliated epithelial cells. Gels containing LuCAFs supported the invasion of NHBECs into the 3D constructs and contracted the collagen gels 2-fold more than gels containing IMR-90 fibroblasts. Masson's trichrome staining indicated collagen-rich fibrotic lesions in the lung tissue from which the primary LuCAFs were derived. Indirect immunofluorescence staining revealed that LuCAFs express alpha-smooth muscle actin (α-SMA), whereas IMR-90 fibroblasts did not express this marker. From these results and observations, we conclude that: 1) Normal fetal fibroblasts support the formation of a well differentiated surface respiratory epithelium in 3D organotypic cultures; and 2) LuCAFs inhibit the differentiation of respiratory epithelial cells and promote their invasion into the 3D collagen gels. A plausible explanation for this invasive behavior is that when compared to IMR-90 fibroblasts, LuCAFs differentially remodel their extracellular matrix and generate an altered stromal microenvironment that provides unique biophysical cues to the overlying epithelial cells, thereby, altering their phenotype.

Title:

Laser Capture Microdissection to Isolate Primary and Metastatic Thyroid Tumors from Formalin Fixed Paraffin Embedded Tissue

Authors:

Caroline Kim and Sheue-yann Cheng

Presented by:

Caroline Kim

Departments:

Division of Endocrinology and Molecular Oncology Research Institute, Tufts Medical Center

Abstract:

Identifying the determinants of thyroid cancer metastasis is often limited by tissue availability. Studies utilizing a mouse model of follicular thyroid cancer (TRßPV mouse) which spontaneously develops thyroid cancer that recapitulates human follicular thyroid cancer behavior, including lung metastases, have identified candidate genes central for thyroid cancer tumorigenesis. Previous microarray analysis on TRßPV mice has not included lung metastatic tumors[1]. Using archived formalin fixed paraffin embedded tissue blocks, we are isolating wild-type, primary thyroid tumors, and metastatic thyroid cancer to lung for eventual microarray analysis. Laser capture microdissection (LCM) of wild-type thyroids, primary thyroid tumors at sites of capsular or vascular invasion, and lung metastases was performed. RNA integrity for each tissue block was assessed by comparing the 3'/5' ratio of the housekeeping gene, ß-actin prior to LCM. As proof-of-principle, quantitative real-time PCR was performed examining the expression level of a known upregulated gene in the thyroids of TRßPV mice, PTTG[1]. Similar to previous reports, PTTG mRNA isolated from laser captured thyroid cancer of TRßPV mice was expressed ~5-fold higher compared to the mRNA from age-matched wild-type thyroids. These findings suggest that LCM of these archived tissue blocks is a viable approach to obtain RNA for microarray analysis. Future studies will compare and contrast the gene expression profiles of primary versus metastatic thyroid tumors that may identify novel contributors to thyroid cancer metastasis.

(1) Ying H., Suzuki H., Furumoto H., Walker R., Meltzer P., Willingham MC., Cheng SY. 2003 Alterations in genomic profiles during tumor progression in a mouse model of follicular thyroid carcinoma. Carcinogenesis 24 (9):1467-79.

Title:

Mrc1 and Tof1 Participate in the Maintenance of CAG•CTG Repeat Tract Integrity

Authors:

Lionel Gellon, Mayurika Lahiri, AnnaLena La Porte, Catherine Freudenreich

Presented by:

Lionel Gellon

Department:

Department of Biology, Tufts University School of Arts and Sciences

Abstract:

Expansion of CAG•CTG repeats is the cause of a number of neurodegenerative diseases. In addition, expanded CAG•CTG repeats have been shown to be fragile sites on a yeast chromosome. Previously, we showed that proteins involved in the S-phase replication checkpoint, which senses stalled forks and induces a checkpoint response, were important for preventing fragility of expanded CAG•CTG repeats (Lahiri et. al, 2004; Freudenreich and Lahiri, 2004). In this study, we have tested the effect of the *S. cerevisiae* Tof1 and Mrc1 proteins, which are known to play a direct role in stabilizing stalled replication forks, on repeat stability. Using a yeast artificial chromosome containing either a CAG•CTG-85 or a CAG•CTG-140 repeat tract or a no repeat control, we show that deletion of either the MRC1 or TOF1 gene resulted in increased repeat tract fragility. In addition, the repeat tract is destabilized in the absence of either protein, resulting in a high level of contractions as well as expansions. Interestingly, complete deletion of MRC1 resulted in a greater rate of fragility as well as frequency of repeat instability compared to checkpoint deficient alleles of MRC1, indicating that the role of Mrc1 in replication fork progression is as important in maintaining CAG•CTG repeats as its checkpoint signaling role. The role of Tof1p appears to be especially crucial at the CAG•CTG-140 tract as repeat fragility and instability was much more pronounced at this longer repeat compared to CAG•CTG-85. Cell growth monitoring showed a significant increase in growth retardation for the Δtof1 strain containing the CAG•CTG-140 tract, and an increase in arrests/swelling for the strains deleted for MRC1. This result corroborates the phenotypes observed in the fragility and instability assays. Thus, these data show that expanded CAG•CTG repeats slow replication fork progression in a eukaryotic system, and that proteins involved in fork restart are very important in maintenance of chromosomal stability at these structure-forming sequences.

Title:

The Importance of Spatial Distribution of Stemness and Proliferation State in Determining Tumor Radio-Response

Authors:

Heiko Enderling, Derek Park, Lynn Hlatky, Philip Hahnfeldt

Presented by:

Heiko Enderling

Department:

Center of Cancer Systems Biology, Caritas St. Elizabeth's Medical Center

Abstract:

Tumor growth and progression is a complex phenomenon dependent on the interaction of multiple intrinsic and extrinsic factors. Necessary for tumor development is a small sub-population of potent cells, so-called cancer stem cells, that live forever, can undergo an unlimited number of cell divisions, and with a small probability divide symmetrically to produce more such cancer stem cells. The majority of a tumor is made up of progeny cancer cells that have a limited life span and a limited replicative potential. Tumor development is dependent on the cancer cell's proliferative behavior, their migratory ability and cell death occurrences. With increasing number of cells in the tumor, competition for space limits tumor progression and the majority of cancer cells becomes quiescent, with proliferation primarily occurring on the outer rim where space is available. We present an agent-based model early tumor development that captures the spatial heterogeneity of proliferating and quiescent cells. We combine the model with the established linear-quadratic model of radiotherapy to predict treatment outcomes for different quiescence radiosensitivities. We show that considering homogeneous radiosensitivity throughout the tumor populations will lead to different results compared to a probable reduced sensitivity of quiescent cells.

Beyond just considering mass effects of stem to non-stem cell ratios and proliferating to quiescent cell ratios, we show that the spatiotemporal evolution of the developing heterogeneous population plays a pivotal role in determining radioresponse and treatment optimization.

Title:

The Yin and Yang of ERK Regulation by Polyomavirus Middle T (MT)

Authors:

Yanni Zhu and Brian Schaffhausen

Presented by:

Yanni Zhu

Department:

Department of Biochemistry, Tufts University School of Medicine

Abstract:

Polyomavirus is noted for its ability to cause tumors in a wide range of tissues. Middle T (MT) is the principal transforming protein of polyomavirus. It is required for viral transformation, and is sufficient to cause tumors when expressed as a transgene. Over the years, MT has provided important insights into mammalian growth control. The importance of tyrosine phosphorylation and phosphoinositide 3-kinase (PI3K) activity were first recognized there.

Our work emphasizes that the effect of MT on cellular signaling of the Ras pathway is conditional. Phosphorylation of MT of Y250 is known to activate the adaptor SHC, that leads to the activation of Ras. This is expected to lead to the activation of Ras effectors including Raf. In turn, this is expected to lead to the activation of ERK1 and ERK2. As expected, MT can activate ERK in MT-transformed cells.

ERK is also strongly activated by stress conditions such as serum starvation, UV radiation, or drugs, e.g., thapsigargin, which triggers the unfolded protein response (also called ER-stress). This can be readily shown with phosphospecific antibodies. In sharp contrast to normal conditions, MT strongly suppresses ERK activation resulting from stress. Examination of other aspects of the UV and unfolded protein response stresses that the MT targets ERK without affecting other aspects of the cellular response.

To better understand how MT suppresses the activation of ERK induced by stress. The canonical ERK activation pathway [MT (Growth Factor)→Adaptor(Shc/Grb2)→SOS→Ras→Raf→MEK→ERK] has been examined and indicated that Ras is activated by MT, whether stress is occurring or not, while MEK activation in response to stress is suppressed. The lost connection between Ras and MEK suggests the regulation of Raf could be the switch to turn off ERK activation by MT. Interestingly, MT315 ($PI3K^-$) is defective on blocking ERK phosphorylation. Inhibition of PI3 kinase by LY294002 partially mimics MT315. Together, this suggests that PI3K or its downstream targets such as AKT is involved in negative regulation of ERK activation by MT under stress. This is an indication of cross-talk between the Ras and PI3-kinase pathways.

Why would a viral protein wish to suppress a stress response? Different kinds of stress (viral infection generating dsRNA, starvation, or as here ER-stress) induce eIF2α phosphorylation to shut down cell protein synthesis. MT also appears to reduce eIF2α phosphorylation under stresses, which would stimulate translation.

Title:

Solution Structure of the Hdlg/SAP97 PDZ2 Domain and its Mechanism for Interaction with HPV-18 E6 Protein

Authors:

Yuqi Liu, Gillian Henry, Rashmi Hegde, James Baleja

Presented by:

Yuqi Liu

Departments:

Department of Biochemistry, Tufts University School of Medicine; Division of Developmental Biology, Cincinnati Children's Hospital Medical Center

Abstract:

High-risk Human Papillomavirus (HPV) E6 proteins, in association with E6AP, can target cellular proteins for degradation, and contribute to the development of cancer. They can bind PDZ domain-containing proteins, including hDlg for proteasome-mediated degradation through their C-terminal PDZ-binding motifs. Our hypothesis is that the complex of E6 and hDlg shows a unique molecular surface that can be used for the assembly with other proteins, and provides a basis for understanding the mechanism of E6-promoted degradation.

The second PDZ domain (PDZ2) from hDlg and a series of short synthesized peptides from C-terminal high-risk HPV E6 were used to mimic the interaction of hDlg and E6. Isothermal titration calorimetry was used to determine the binding affinities. The solution structure of PDZ2 in complex with HPV-18 E6 peptide was determined by NMR with backbone root-mean-square deviations of less than 0.5Å. The structure shows a typical PDZ architecture containing six β-strands and two α-helices. The C-terminal six residues of E6 form a short β-strand that binds in a groove formed by αB and βB and anti-parallel to βB strand. However, a novel mode of interaction was found in which six residues of the HPV-18 E6 peptide are contacted by the PDZ2 domain, in contrast to the typical four residues used by PDZ domains.

Molecular dynamics simulations support a model in which the C- and N-terminal ends of the peptide have different mobilities within the complex. Comparison of the NMR complex structure to previously determined X-ray structures of PDZ2 by itself and bound to different peptides allows a description of conformational changes required for PDZ2 to bind to HPV-18 E6. The binding of E6 peptide with loop 2 of PDZ2 induces additional contacts between loop 2 and the αB helix, pulls the C-terminus of αB inside and makes the PDZ2 domain more compact.

Title:

Scaffold Proteins Regulate Localized Rac Activation by Tiam1

Authors:

Soumitra Rajagopal, Yuhuan Li, Kathleen Wicks, Ka-Wing Wong, Ralph Isberg, Rachel Buchsbaum

Presented by:

Soumitra Rajagopal

Departments:

Molecular Oncology Research Institute and Department of Medicine, Tufts Medical Center; Department of Molecular Biology and Microbiology, Tufts University School of Medicine; Department of Microbiology and Immunology, Albert Einstein College of Medicine; Boston Medical Center; Oregon State University

Abstract:

Tiam1 is a widely expressed exchange factor for the Rac GTPase with effects on multiple cell functions including growth, apoptosis, polarity, and motility. We have previously found that Tiam1 interaction with different scaffold proteins leads to activation of different specific pathways downstream of Rac due to recruitment of specific Rac effector proteins to Tiam1-scaffold complexes. Tiam1 is known to interact with a number of proteins and many of these interactions are mediated through the same N-terminal region of the protein containing a pleckstrin homology (PH) domain immediately upstream of a coiled-coil (CC) domain. This raises the question of how signaling specificity is achieved. We reasoned that there must be regulatory mechanisms governing the specific conditions under which each interaction occurs. We hypothesized that Tiam1-interaction with one scaffold complex might lead to Tiam1-mediated Rac activation in response to one upstream signal, while interaction with another scaffold complex would lead to Tiam1-mediated activation in response to another upstream signal. Fibroblasts expressed at least two Tiam1-interacting scaffold proteins, IRSp53 and spinophilin. We used fluorescent resonance energy transfer (FRET) to measure localized Rac activation in association with IRSp53 and spinophilin complexes in individual fibroblasts as a way to test this hypothesis. We found that treatment with pervanadate or PDGF induced localized Rac activation was dependent on both Tiam1 and IRSp53, but not spinophilin. Treatment with forskolin or epinephrine induced localized Rac activation was dependent on both Tiam1 and spinophilin, but not IRSp53. In contrast to the localized Rac activation seen using the FRET assay, overall levels of activated Rac in cell lysates did not change with any treatment. In addition, downstream effects of Rac activation were stimulus and scaffold-specific. Pervanadate-induced ruffling and membrane translocation of Tiam1 were dependent on IRSp53, but not spinophilin. IRSp53, but not spinophilin, was required to maintain Tiam1-dependent cell adhesion. Epinephrine stimulation led to decreased IRSp53-dependent adhesion in a Rac and spinophilin-dependent fashion. Taken together, these results support the idea that interactions of Tiam1 with multiple different scaffold proteins couple the transmission of distinct upstream signals to localized Rac activation, with subsequent activation of specific downstream pathways. In addition, our findings suggest that manipulating the interactions of exchange factors such as Tiam1 with scaffold protein complexes can modulate cellular behaviors involving Rac activation.

Title:

Sequence-Specific Chromatin Remodeling of the C-Myc Promoter by hSWI/SNF

Authors:

Hillel Sims, Cassandra Baughman, Jacqueline Lane, Gavin Schnitzler

Presented by:

Gavin Schnitzler

Departments:

Departments of Biochemistry and of Anatomy and Cellular Biology, Tufts University School of Medicine

Abstract:

Increased transcription of the protooncogene c-myc is associated with more than half of all human tumors. One factor that is important for c-myc transcription is the ATP-dependent chromatin remodeling complex, human SWI/SNF (hSWI/SNF). This suggests that chromatin changes introduced by hSWI/SNF will activate transcription by changing the accessibility of the c-myc promoter to transcription factors. The precise nature of these chromatin changes and the role of c-myc promoter sequence in directing them, however, is unknown. Here, we examine the effects of hSWI/SNF on c-myc promoter chromatin templates. Our results show that, before remodeling, two favored nucleosome positions exist on c-myc. These positions, which are also observed on the inactive promoter *in vivo*, block access to many important transcription factor binding sites. Strikingly, in a single nucleosome system, hSWI/SNF responds to c-myc promoter sequence to quantitatively move nucleosomes away from these default repressive positions. In a multinucleosome system, hSWI/SNF moves these nucleosomes in a somewhat different way, pushing them together to form normal or structurally-altered dinucleosomes. Overall, our results indicate that hSWI/SNF alters chromatin in a promoter-specific manner, and provide new insights into the mechanism of c-myc transcriptional regulation.

Title:

Inhibition of CBF-1-Mediated Notch Signaling Induces FGF-Dependent Transformed Cell Phenotype

Authors:

Doreen Kacer, Christian McIntire, Alexander Kirov, Igor Prudovsky

Presented by:

Igor Prudovsky

Department:

Center for Molecular Medicine, Maine Medical Center Research Institute

Abstract:

Notch signaling is an evolutionarily conserved regulatory pathway, which is involved in cell proliferation, differentiation and cell survival decisions. Once the Notch receptor is bound by the ligand, the intracellular domain is detached by γ-secretase and is translocated to the nucleus where it acts as a transcriptional regulator of the CBF-1-dependent Notch signaling cascade. Our laboratory previously demonstrated that soluble forms of Notch ligands, such as soluble Jagged 1, induce the expression and stress-independent non-classical release of FGF1 (D. Small et al, J. Biol. Chem, 2003, v. 278, pp. 16405-16413). This effect correlated with the inhibition of Notch signaling. We suggested that downregulation of Notch signaling in damaged tissues could stimulate the expression and release of FGF1, which is required for tissue repair. However, we did not have direct proof of the hypothesis that the inhibition of Notch signaling induces FGF1 transcription and export. The **aims** of the present work are: 1) To verify the hypothesis about the role of Notch signaling in FGF1 release and expression using the dominant negative (dn) form of CBF-1, a key transcription factor of the Notch signaling cascade; and 2) To explore the cell phenotype induced by the inhibition of the CBF-1-dependent Notch signaling.

NIH 3T3 cells were stably transfected with dnCBF-1 and examined for the following characteristics: (i) FGF1 expression (using RT-PCR); (ii) FGF1 release (using adenoviral transduction of FGF1 followed by heparin chromatography of conditioned media, SDS-PAGE, and Western blotting); (iii) growth rate; (iv) ability to grow in soft agar; and (v) tumor formation in nude mice. DnCBF-1 expression resulted in the appearance of a detectable level of the FGF1 transcript. Unlike control cells, dnCBF-1 transfectants released FGF1 at non-stress conditions. dnCBF-1 transfectants exhibited a loss of growth contact inhibition, anchorage independent growth in soft agar, and rapid formation of highly angiogenic tumors in nude mice. Significantly, the inhibition of FGFR signaling and of the function of S100A13, a key component of FGF1 export pathway, resulted in a drastic inhibition of anchorage independent growth of dnCBF-1 cells and restored growth contact inhibition. Interestingly, DAPT, inhibitor of g-secretase and Notch signaling considered for the treatment of Alzheimer disease, strongly enhanced the growth of NIH 3T3 cells, and this effect was FGFR-dependent. We suggest that the export of FGF1 induced as a result of Notch signaling inhibition contributes to the development of a transformed cell phenotype and to enhanced angiogenesis in tumors.

Title:

Investigating Cancer Progression of Cells in 3D Matrix with Non-Invasive Fluorescent Imaging

Authors:

Joanna Xylas, Addy Alt-Holland, Jonathan Garlick, Irene Georgakoudi

Presented by:

Joanna Xylas

Departments:

Department of Biomedical Engineering, Tufts University School of Engineering; Departments of Endodontics and of Oral and Maxillofacial Pathology, Tufts University School of Dental Medicine

Abstract:

Epithelial cell adhesion to adjacent cells and matrix is crucial to invasive potential and influences cell organization and behavior. It is unclear what triggers cancer cells to lose these abilities and the relation to metastasis onset. Since most cancers can be cured if detected before they attain strong invasive capacity, understanding and detecting the changes that occur in the premalignant-to-malignant transformation would significantly advance the medical field.

Our study aims to investigate how genetic makeup and cell local environment influence metastatic behavior with two-photon excited fluorescence (TPEF) and second harmonic generation (SHG). By exploiting natural non-linear fluorescence and scattering properties of cell and matrix, these techniques have potential to non-invasively characterize tissues as well as develop a method to directly assess these optical biomarkers of cancer in real time.

We created a model consisting of collagen type-I, human fibroblasts, and *ras*-transformed human epidermal keratinocytes (HEKs) or *ras*-transformed, E-cadherin-deficient HEKs as benign or malignant cell equivalents, respectively. Our data indicate that upon E-cadherin suppression and loss of cell-cell contact, E-cadherin-deficient tumor cells migrate into surrounding matrix as single cells. E-cadherin-competent cells are organized in rounded, compact clusters that expand evenly in all directions. However, upon incorporation of fibroblasts into the collagen gels, we observe significant enhancements in the degree E-cadherin-competent cell migration, suggesting that environmental factors also play a significant role in cell migration and potentially metastasis. Fourier-based image analysis approaches indicate that an inverse power law dependence characterizes the power spectral density (PSD) of the intensity fluctuations of the TPEF images over a range of spatial frequencies, indicating differences in the benign and malignant cell features on the micrometer scale.

We expect these optical biomarkers to provide a foundation for non-invasive clinical evaluation of the malignant potential of precancerous cells.

Title:

Cancer Cells Modulate DNA DSB/Repair in Nontransformed Cells

Authors:

Afshin Beheshti, Heiko Enderling, Matthew Perkins, Aaron Burg, Philip Hahnfeldt, Lynn Hlatky

Presented by:

Afshin Beheshti

Department:

Center of Cancer Systems Biology, Caritas St. Elizabeth's Medical Center

Abstract:

Gamma-H2AX (phosphorylated H2AX from the core histone H2AX) and 53BP1 (p53 binding protein 1) co-localize at sites of DNA damage with a number of proteins involved in DNA damage repair and signaling, thus facilitating DSB repair and rejoining. Gamma-H2AX and 53BP1 play a critical role in suppressing oncogenic translocations. Although induction of Gamma-H2AX foci and 53BP1 foci are frequently utilized as assays to investigate DSB/repair following irradiation, minimal attention has been given to the generation of spontaneous DSB/repair foci in cancer cells and cells of the tumor microenvironment. We investigated the constitutive level of DNA damage/repair, as indicated by standard Gamma-H2AX and 53BP1 foci assays, in cells of the tumor and adjoining stroma *in vivo. In vitro* studies were also done on a panel of murine tumor cells (lung carcinoma) and human tumor cells (lung carcinoma), in both direct and indirect co-cultures with primary nontransformed cells (murine lung fibroblast, human dermal fibroblast). *In vivo* studies on a panel of tumor models, including human tumor xenografts (liposarcoma), murine tumors (Lewis lung carcinoma), and the spontaneous K-ras LA2 lung tumor model were also performed. DSB repair foci in both *in vitro* and *in vivo* tumor stromal populations were quantified. *In vivo* spatial distributions were compared to other measured tumor features (e.g. vascularization, oxygenation, etc). An increase in DSBs occurred in nontransformed cells in the presence of tumor cells *in vitro*. Conversely, preliminary data suggest that stromal fibroblasts may induce a reduction of DSB foci in the tumor cells. In the *in vivo* tumor models, gamma-H2AX and coupled 53BP1 expressions were detected in several cell types (e.g., endothelial, fibroblasts, adipocytes, etc.) in the adjacent tumor microenvironment. At distances away from the tumor, these same cell types, showed null or minimal levels of gamma-H2AX and 53BP1. Our studies demonstrate, both *in vivo* and *in vitro*, that tumor cells can induce DSB/repair foci in adjacent nontransformed cells. This suggests that tumor cells may transmit signals into the microenvironment that result in DNA damage to neighboring nontransformed cells. We termed this effect the "constitutive-break bystander effect."

Title:

Antiangiogenic Effects of Proton Irradiation

Authors:

Swati Girdhani, Philip Hahnfeldt, Afshin Beheshti, Zachary Anaya, Michael Peluso, Clare Lamont, Raktima Raychowdhury, Christian Schwager, Peter Huber, Heiko Enderling, Amir Abdollahi, Lynn Hlatky

Presented by:

Swati Girdhani

Departments:

Center of Cancer Systems Biology, Caritas St. Elizabeth's Medical Center; Department of Radiation Oncology, German Cancer Research Center (DKFZ), Germany

Abstract:

Tumor angiogenesis, i.e., the recruitment of microvascular endothelial cells to the tumor site, has emerged as an important target in cancer therapy. How radiation therapy modulates tumor angiogenesis, and conversely, how angiogenesis factors modulate cell radio-response, is a central question for the optimization of radiation treatment. The superior physical characteristics of dose delivery by proton- vs. conventional photon-radiotherapy have attracted considerable attention in clinical oncology. This is evidenced by increasing numbers of proton facilities being built worldwide. In contrast to the physical aspects of proton radiation, which are heavily exploited, the radiobiological, molecular and cell-level responses triggered by proton (vs. photon) radiations are understudied and thus underexploited.

We demonstrate that proton radiation (at least at higher energies) is antiangiogenic and this advantageous biological aspect of protons should be further explored to optimize treatment efficacy. We report that proton irradiation inhibits major pro-angiogenic factors in a dose-dependent manner in both the tumor and tumor-microenvironment (tumor-stroma and microvasculature). Differentially regulated genes 6h after 0, 0.5, 1 and 2 Gy proton irradiation were detected in human dermal fibroblasts and human lung microvascular endothelial cells (HMVEC-L) using pan-genomic human microarrays. Of note, critical pathways in pro-angiogenic signaling such as vascular endothelial growth factor (VEGF), interleukin 6 and 8 (IL6, IL8) and the hypoxia inducible factor 1α (HIF-1α) were significantly downregulated after proton irradiation in both cell types. The proton induced dose and time dependent downregulation of these pro-angiogenic genes were confirmed by real time quantitative RT-PCR and ELISA. To investigate the regulation of these genes in tumor cells, their expression was tested in human non-small cell lung cancer cell line A549 and mouse Lewis lung carcinoma cells (LLC). Both tumor cell lines showed similar patterns of downregulation of angiogenic genes on RNA and protein levels. Additionally, we investigated the effects of proton irradiation on cell invasion/migration using the matrigel invasion assay. It was found that proton irradiation decreased cell invasion in all cell lines tested. To functionally validate the role of VEGF and IL8 paracrine signaling in proton induced anti-angiogenic and anti-invasive effects, *in vitro*, co-culture models were used. Importantly, addition of recombinant IL8 or VEGF into

the media partially rescued the cells from the proton radiation induced anti-invasion effects, suggesting a functional role for these factors. Furthermore, proton-irradiated A549 cancer cells, exhibited delayed growth *in vivo,* demonstrating that radiation-induced interactions with host tissues can slow the transition through critical carcinogenesis bottlenecks. These findings suggest that proton irradiation suppresses the regulation of key pro-angiogenic and pro-invasive proteins such as VEGF and IL8 at clinically relevant doses. The antiangiogenic and anti-invasive effects of proton irradiation demonstrated here provide novel preclinical evidence for beneficial radiobiological effects of proton vs. photon radiations that may be exploited clinically.

Title:

Impaired Angiogenesis in Aged Tumor Microenvironment Attenuates Tumor Growth

Authors:

Shiva Kalinga, Afshin Beheshti, Philip Hahnfeldt, Amir Abdollahi, Lynn Hlatky

Presented by:

Shiva Kalinga

Department:

Center of Cancer Systems Biology, Caritas St. Elizabeth's Medical Center

Abstract:

The incidence of cancer increases progressively with age in both animals and humans, however, there are no uniformity patterns of age-related distribution of tumors. To study age-dependent induction of tumor in mice, we injected carcinomic human alveolar basal epithelial cells (A549) in nude mice in the age group of 3-12 months. Preliminary results suggest that in aged animals the tumor growth is repressed to certain extent and the development of tumor in young mice is more prominent compared to older mice. In addition, we observed similar tumor growth kinetics after exposing nude mice to 0.2 Gy iron radiation. Two of the several hypotheses will be discussed: 1) levels of angiogenesis expression in both young and old mice, and 2) age-induced expression of tumor suppressor genes such as P53 and p16INK4a.

Title:

Neuregulin-1 Alleviated Doxorubicin-Induced Down-Regulation of Cardiac Troponin Proteins in the Heart

Authors:

Yun Bian, Maoyun Sun, Marcy Silver, Kalon Ho, Mark Marchionni, Anthony Caggiano, James Morgan, Xinhua Yan

Presented by:

Xinhua Yan

Departments:

Division of Cardiovascular Research, Caritas St. Elizabeth's Medical Center; Cardiovascular Division, Beth Israel Deaconess Medical Center; NRG Biotech; Acorda Therapeutics Inc.

Abstract:

Chemotherapy-induced cardiotoxicity is a major hurdle in cancer therapy. Recently developed new cancer therapy targeting the erbB2 receptor by Trastuzumab significantly reduced the recurrence and early mortality in breast cancer patients. Yet, when Trastuzumab was used in combination with the chemotherapy drug, doxorubicin, the incidence of class III and IV heart failure increased nearly 20%.

Previous studies form our lab and others showed that Neuregulin-1 (NRG1) was effective in protecting the heart from doxorubicin. Since NRG1 activates the erbB2 receptor on cardiomyocytes, understanding how NRG1 provides cardioprotective effects from doxorubicin is crucial for developing a safe and successful therapy in the clinical setting.

In this study, by using an *in vivo* doxorubicin cardiotoxicity mouse model, we demonstrated that: 1) NRG1 improved both survival and cardiac function in doxorubicin-injured mice, and 2) doxorubicin caused down-regulation of multiple thin filament proteins in the heart; NRG1 selectively maintained troponin proteins but not others. Further, we used neonatal rat ventricular myocytes (NRVM) to study how NRG1 preserved cardiac troponin proteins. We demonstrated that NRG1 inhibited doxorubicin-induced down-regulation of the mRNA levels of cardiac troponin I (cTnI) and cardiac troponin T (cTnT), as well as doxorubicin-induced deactivation of the mTOR pathway. Doxorubicin activated multiple caspases that were responsible for the decreasing of cTnI and cTnT. Doxorubicin also increased proteasome degradation of cTnI. NRG1 inhibited these effects of doxorubicin. These effects of NRG1 depended on the erbB2 receptor, as well as the PI3K, Akt and mTOR pathways, but not by the erbB4 receptor, PKC or p38. These results demonstrated that NRG1 restored the levels of cTnI and cTnT by increasing the transcription and translation, as well as by decreasing caspase activation and proteasome degradation of these proteins.

This study provided new experimental evidence on how NRG1, via the erbB2 receptor, protects the heart from chemotherapy-induced heart failure. A clearer understanding of how the NRG-erbB pathway regulates survival signaling in diseased hearts will contribute to the development of more efficacious and safer therapies for chemotherapy-induced heart failure, and also, to the improvement of overall health of cancer patients.

Title:

Evaluating the Efficacy of Stereotactic Spinal Radiosurgery

Authors:

Kevin Yao and Debbie Bakes

Presented by:

Kevin Yao

Department:

Boston Institute of Neurosurgery, Tufts Medical Center

Abstract:

Hypothesis: Spinal radiosurgery offers an important new therapeutic modality for the treatment of spinal metastases and represents an extension of the current state-of-the-art radiation therapy. The purpose of this study was to evaluate the accuracy and efficacy of spine metastasis radiosurgery. Over the past several years, literature reviews have anecdotally suggested that radiosurgery for spine metastases can be effective for pain management, tumor control, and maintaining neurological function. However, it is unclear who are the most appropriate patients to be treated, and what are the optimal radiation planning and dosing regimens. By creating and periodically reviewing a prospective clinical database of spine radiosurgery treatments, we hope to critically address these issues.

Materials and Methods: The initial portion of this study involved the creation of a prospective clinical database as to collect and organize the records of patients treated using single-fraction or hypofractionated radiosurgery for spinal metastases. Data include patient demographics, tumor histology, description of patient immobilization, treatment planning, and treatment delivery. The second portion of this study involves periodic evaluation of the database to critically assess clinical efficacy of radiosurgery treatment for spinal metastases. Outcome parameters include pain relief, local and systemic tumor control, and neurological function.

Results and Discussion: To date the database includes five patients with spinal metastases who were treated with image guided radiosurgery at Tufts Medical Center. Four of 5 (80%) patients have shown pain improvement while all patients are either stable or improved neurologically. All patients are alive with stable disease. Five out of 5 (100%) treated tumors have demonstrated radiographic tumor control at last follow-up. The mean follow-up period was 2.5 months (range, 1.5-4 months), with a median follow-up of 2.25 months. This preliminary data suggest not only that radiosurgery is feasible and safe in the treatment of spinal metastases, but also that excellent tumor control, pain relief, and preservation of neurological function can be achieved.

Title:

Selective Inhibition of Growth of Tuberous Sclerosis Complex 2-Null Cells by Atorvastatin is Associated with Impaired Rheb and Rho Gtpase Function and Reduced Mtor/S6 Kinase Activity

Authors:

Geraldine Finlay, Amy Malhowski, Yinglin Liu, Barry Fanburg, David Kwiatkowski, Deniz Toksoz

Presented by:

Deniz Toksoz

Departments:

Department of Medicine, Tufts Medical Center; Department of Medicine, Brigham and Women's Hospital

Abstract:

Inactivating mutations in the tuberous sclerosis complex 2 (TSC2) gene, which encodes tuberin, result in the tumorigenic conditions of TSC and lymphangioleiomyomatosis (LAM), which are ultimately fatal. The tumor suppressor effect of tuberin lies in its GTPase-activating protein (GAP) activity toward Rheb small GTPase, a Ras superfamily member. The statins, HMG CoA reductase inhibitors, have pleiotropic effects which may involve interference with Ras and Rho GTPase prenylation and function. We show that atorvastatin selectively inhibits serum- and estrogen-induced proliferation of Tsc2-/- fibroblasts and smooth muscle cells. The isoprenoids farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) significantly reverse atorvastatin-induced Tsc2-/- cell growth inhibition, suggesting that atorvastatin dually targets a farnesylated protein, such as Rheb, and a geranylgeranylated protein, such as Rho, both of which are activated in Tsc2-/- cells. Atorvastatin reduced Rheb isoprenylation, GTP loading, and membrane localization. Atorvastatin also inhibited the constitutive phosphorylation of mTOR, S6 kinase, and S6 found in Tsc2-/- cells in an FPP-reversible manner, and attenuated the high levels of phosphorylated S6 in Tsc2-heterozygous mice. Atorvastatin, but not rapamycin, attenuated the increased levels of activated RhoA levels in Tsc2-/- cells, and this was reversed by GGPP. These results suggest that atorvastatin may inhibit both rapamycin-sensitive and rapamycin-insensitive mechanisms of tuberin-null cell growth, at least in part via Rheb and Rho inhibition, respectively. Atorvastatin may have potential therapeutic benefit in TSC syndromes, including LAM.

Title:

Genetic Variants in Uracil Processing Enzymes are Associated with Abnormal DNA Uracil Content

Authors:

Aurelie Chanson, Larry Parnell, Jimmy Walter Crott, Sang Woon Choi, Haewook Han, Joel Mason

Presented by:

Aurelie Chanson

Departments:

Vitamins and Carcinogenesis Laboratory and Genomics Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University; General Clinical Research Center, Tufts Medical Center

Abstract:

Introduction: Maintenance of DNA integrity through DNA repair is critical in cancer prevention. Among the enzymes involved in DNA repair are those that prevent or correct uracil misincorporation in DNA. Repair of misincorporated uracil is important since it can cause double-stranded DNA breaks and other mutagenic events that can initiate or promote carcinogenesis. Also relevant is the B-vitamin folate as it is a co-factor in the enzymatic conversion of uracil to thymidine and its deficiency promotes uracil misincorporation in DNA. Moreover, folate and other B vitamins are implicated as factors modulating cancer risk. Five genes encode the enzymes involved in uracil-repair in humans, for which many single nucleotide polymorphisms (SNPs) have been identified. However, their phenotypes have not yet been explored. Our goal is to identify SNPs in uracil processing genes that are determinants of DNA uracil levels, and to study how B vitamin status affects these relationships.

Methods: Twenty-two SNPs were selected with a bioinformatics approach that identified variants with probable functional consequences and from distinct linkage groups. Association between SNP genotypes and uracil levels in blood DNA was tested in a cohort of 91 unrelated South Koreans.

Results: Multiple linear regression analyses showed that five SNPs in two of the uracil-repair genes were significantly associated with uracil levels. Four of them were associated with an increase in uracil in the homozygous variant individuals compared to the homozygous wild-type subjects and one was associated with a decrease (see table below). Four SNPs were located in non-coding regions whereas one was a non-synonymous SNP within an exon that is predicted to change the protein sequence. No interactions between B vitamin levels, SNP genotypes and uracil levels were found in this study.

Conclusion: We identified five SNPs with a biochemical phenotype that may impact on cancer risk. We are presently confirming our observations in a larger study.

Details of the five SNPs in the two uracil-repair genes found to be associated with DNA uracil levels:

Gene/SNP Designation	**SNP Type/Location**	**ΔUracil[1]**	**Adjusted *P* Value[2]**
MBD4/rs140693	exon, non-synonymous	1.84-fold increase	0.011
MBD4/rs2005618	intron 6, Tag SNP	2.07-fold increase	0.033
MBD4/rs4128193	3'-UTR (downstream)	2.42-fold increase	0.006
MBD4/rs9821282	promoter	2.38-fold increase	0.007
UNG/rs1018783	promoter	1.55-fold increase*	0.038

* No aa individuals, so the reported value is for Aa vs AA individuals
[1] aa vs AA individuals (a: variant allele, A: wild-type allele)
[2] adjusted for homocysteine, gender, serum creatinine, B vitamin status

Title:

Dipeptidyl Peptidase 2 is a Novel Prognostic Factor and Therapeutic Target in Chronic Lymphocytic Leukemia

Authors:

Alexey Danilov, Olga Danilova, Andreas Klein, Jennifer Brown, Arthur Rabinowitz, Kenneth Miller, Brigitte Huber

Presented by:

Alexey Danilov

Departments:

Division of Hematology/Oncology, Tufts Medical Center; Center for Hematologic Oncology, Dana Farber Cancer Institute; Department of Hematology and Oncology, Lahey Clinic

Abstract:

Chronic lymphocytic leukemia (CLL) is a malignancy with heterogeneous outcome. Expression of CD38 and ZAP-70 and low rate of IgVH mutations correlate with adverse outcome in CLL. In our lab, we have cloned a serine protease, Dipeptidyl peptidase 2 (DPP2), which is implicated in maintenance of quiescence in normal lymphocytes. Inhibition of DPP2 leads to apoptosis of normal resting cells. We previously reported resistance of CLL cells to DPP2 inhibition-induced apoptosis in ~40% of CLL cases, which correlated with an adverse clinical outcome. Here, we confirm our initial observation on a large cohort of patients and demonstrate strong correlation with IgVH mutation status. We also demonstrate that inhibition of hsp90 may restore DPP2 sensitivity to resistant CLL.

The patient cohort included 140 subjects with B-CLL from the Hematology clinics at Tufts Medical Center, the Dana-Farber Cancer Institute (both in Boston, MA), and the Lahey Clinic (Burlington, MA). Median follow up from diagnosis in the study was 6 years. Forty seven patients (33.6%) received at least one treatment during the course of their disease. CLL B-cells were isolated from peripheral blood with standard Ficoll-Hypaque technique, treated with ValboroPro (VbP, Point Therapeutics), a non-specific inhibitor of DPPs, and AX8819 (ActivX), a DPP2-specific inhibitor, incubated for 16 hours and stained with anti-CD19 antibodies, propidium iodide and Annexin V. Cells were treated with 17-Allylaminodemethoxygeldanamycin (17-AAG, Calbiochem), an inhibitor of hsp90 (a protein involved in stabilization of ZAP-70), or with R-406, a Syk kinase inhibitor. IgVH mutational status was available for 44 samples. ZAP-70 expression by flow cytometry was available for 55 samples.

Inhibition of DPP2 with either VbP or AX8819 resulted in CLL cell apoptosis in 93 cases (66.4%). The remaining CLL cases were resistant to both agents (R-CLL). In the R-CLL subgroup, 32 patients required treatment for their disease (68.1%), contrary to S-CLL, where 22 patients initiated treatment (23.7%). Patients with R-CLL required treatment earlier than S-CLL (p=0.03). Among S-CLLs, 91% had a mutated IgVH, 76%

expressed low ZAP-70 and 82% low CD38. Among R-CLL, 91% of samples had an unmutated IgVH, 100% expressed high ZAP-70 but only 45% high CD38.

Concomitant treatment of B-cells with AAG-17 and AX8819 increased apoptosis by an average of 8.1% in R-CLL, but not in S-CLL. R-406 alone caused 14% death in S-CLL and 6% in R-CLL. R406 displayed a synergistic effect with AX8819, causing 7% death in S-CLL and 5% in R-CLL samples, in addition to that caused by AX8819.

DPP2 inhibition discriminates two subsets of CLL based on their ability to undergo apoptosis upon disruption of the quiescent program. Patients with R-CLL have worse disease outcome, with unmutated IgVH and expresses high ZAP-70 levels, in contrast to S-CLL, which have mutated IgVH and low ZAP-70 expression. Inhibition of SYK kinase enhances apoptosis in both subgroups. Whereas, decreasing the amount of stable ZAP-70 with 17-AAG, an hsp90 inhibitor does not affect apoptosis in S-CLL, it partially reverses the phenotype in some R-CLL samples, implicating a role for ZAP-70 or other stabilization-dependent factor in resistance to apoptosis in CLL.

Title:

Distinct Gab2-Mediated Signaling Pathways are Essential for Myeloid or Lymphoid Transformation and Leukemogenesis by BCR-ABL

Authors:

Wayne Chan, Golam Mohi, Shaoguang Li, Benjamin Neel, Richard Van Etten

Presented by:

Wayne Chan

Departments:

Departments of Physiology and of Medicine, Tufts University School of Medicine; Molecular Oncology Research Institute, Tufts Medical Center; Department of Pharmacology, SUNY Upstate Medical University; Department of Medicine, Harvard Medical School; The Jackson Laboratory

Abstract:

The BCR-ABL oncogene encodes an activated fusion tyrosine kinase that causes chronic myelogenous leukemia (CML) and B-lymphoid acute lymphoblastic leukemia (B-ALL) in humans. An autophosphorylation site at Tyr177 of BCR-ABL recruits Grb2 via its SH2 domain and is required for efficient induction of CML-like myeloproliferative disease by BCR-ABL in a mouse BM retroviral transduction/transplantation model. We previously showed (Sattler et al., Cancer Cell 2002; 1:479) the scaffolding/adapter protein Gab2 is recruited to Y177 of BCR-ABL via a Grb2/Gab2 complex and *in vitro* transformation of primary myeloid and lymphoid progenitors by BCR-ABL was impaired in bone marrow from mice with homozygous null mutations in the Gab2 gene ($Gab2^{-/-}$ mice), coincident with decreased activation of the ERK and Akt signaling pathways. Here, we demonstrate an essential requirement for Gab2 in myeloid and lymphoid leukemogenesis by BCR-ABL. Whereas, recipients of BCR-ABL-transduced $Gab2^{+/+}$ BM develop fatal CML-like myeloproliferative disease within 4 week of transplantation, recipients of BCR-ABL-transduced $Gab2^{-/-}$ BM fail to develop CML but succumb after a long latent period to T-cell acute lymphoblastic leukemia, phenocopying the disease induced by the BCR-ABL Y177F mutant. These results suggest that the Y177F and Gab2 mutations have an epistatic relationship and that the critical transforming signals from Tyr177 of BCR-ABL are transmitted through Gab2. Co-expression of Gab2 with BCR-ABL in $Gab2^{-/-}$ BM restored efficient induction of CML-like leukemia, but mutants of Gab2 that lacked either the pleckstrin homology domain or Tyr binding sites for the SH2 domains of the downstream Gab2 effector molecules SHP2 or p85 PI3K failed to rescue myeloid leukemogenesis by BCR-ABL, although the mutant Gab2 proteins were expressed in circulating myeloid cells. Gab2 deficiency attenuated B-lymphoid transformation by BCR-ABL in vitro, and significantly prolonged the latency of B-ALL induced by BCR-ABL in mice. In contrast to CML, induction of B-ALL in $Gab2^{-/-}$ BM was rescued by either WT Gab2 or the Gab2 p85 binding site mutant. These results demonstrate that BCR-ABL absolutely requires signaling via Gab2 to both SHP2 and PI3K to cause CML, while a Gab2–SHP2 signaling pathway contributes to the pathogenesis of $BCR\text{-}ABL^{+}$ B-ALL.

Title:

Environmental Risk Factors and Risk of Canine Malignant Lymphoma: A Model for Human Non-Hodgkin's Lymphoma

Authors:

Sofija Zagarins, Lisa Barber, Elizabeth Procter-Gray, Audra Gollenberg, Elizabeth Bertone-Johnson

Presented by:

Lisa Barber

Departments:

Department of Clinical Sciences, Cummings School of Veterinary Medicine; Department of Public Health, School of Public Health and Health Sciences, University of Massachusetts Amherst

Abstract:

Epidemiologic studies of companion animals offer an important opportunity to identify risk factors for cancers of relevance to both animals and humans. Canine malignant lymphoma (CML) has been established as a model for non-Hodgkin's lymphoma (NHL) and previous studies have suggested that exposure to environmental chemicals such as 2,4-dichlorophenoxyacetic acid (2,4-D) may relate to the development of CML. We conducted a case-control study of dogs presented to the Foster Hospital at Tufts University between 2000 and 2006. Cases were 263 dogs with biopsy-confirmed CML. Controls included 240 dogs with benign tumors and 230 dogs undergoing surgeries unrelated to cancer. Dog owners completed a 10-page questionnaire measuring demographic, environmental, and medical factors. After adjusting for age, weight, and other factors, we did not find either household smoking or use of flea/tick control products to be associated with risk of CML. Use of professionally-applied lawn care products was positively associated with CML risk. In particular, herbicide use was associated with a significant 50% higher risk of CML [odds ratio (OR): 1.5; 95% confidence interval (CI): 1.1-2.3], while pesticide use was associated with an 80% higher risk (OR: 1.8; 95% CI: 1.2-2.9). Home owner application of these chemicals was unrelated to risk, though the OR for use of insect growth regulators was 2.7 (95% CI: 1.1-6.9). These findings suggest that some lawn care chemicals may increase the risk of CML. Additional analyses are needed to evaluate the specific chemical components of these products, such as 2,4-D, that may be related to risk of CML and perhaps to human NHL.