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GS 1 - GENERAL SESSION 1 – PATHOLOGY AND MOLECULAR PROFILING

GS1.1
INSIGHTS INTO BREAST CANCER-RELATED GENE NETWORKS THROUGH CROSS-SPECIES COMPARISONS

Green J. E.
Laboratory of Cell Regulation and Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

Genomic analyses of breast cancer have demonstrated that histologically similar tumors are quite distinct on a molecular level, which has important implications for prognosis and therapy. Defining these molecular characteristics on a global scale using expression profiling provides a means for learning how oncogenic molecular pathways are integrated and identifying elements that could be important targets for therapeutic intervention. Confounding this approach, however, is the relatively large degree of genetic heterogeneity in the human population that may obscure the recognition of important differences in gene expression and gene interactions. In order to improve the identification of critical genes and networks involved in determining whether a tumor is hormone-responsive or whether a tumor expresses an aggressive proliferative and metastatic constellation of genes, we have used evolutionary conservation of gene expression in mammary tumors as a window to identify critical genes and pathways. Comparing gene expression patterns in human breast tumors with that of tumors generated in several genetically-engineered mouse models of mammary cancer has revealed that certain models can be classified with certain sub-types of breast cancer based upon the cell-type of origin of the cancers and whether they possess a specific proliferation/DNA-damage signature indicating a poor prognosis. Additionally, a novel approach of combining both mouse and human microarray data led to the identification of a set of genes related to the ER status and, therefore, hormone dependence of mammary tumors. The cross-species approach has led to further insights into molecular events involved in both human and mouse and has important implications for the use of genetically-engineered mouse models for the study of human breast cancer.

GS1.2
MAMMARY PRECANCERS: OLD CONCEPT AND NEW BIOLOGY

Cardiff R.D.[1] and Muller W.J.[2]
[1]*University of California, Davis, Davis, CA, USA;*
[2]*McGill University, Montreal, QC, Canada*

A century ago (1906), Apolant described hyperplastic nodules (HAN) in mouse mammary glands. Haaland (1911) associated HAN wtih spontaneous mouse mammary cancer. Ewing (1911) first described human breast precancers. Subsequently, numerous mouse-human comparisons were attempted. Dunn (1954) summarizing breast precancers:

> *"Attempts have been made to correlate the histologic appearances in the mammary tissue of old female mice of high cancer strains, with supposedly precancerous conditions in women. Such attempts have been much hampered by the fact that there has been no clear agreement as to what constitutes a precancerous condition in either species. It is an attempt to check one unknown against another unknown."*

DeOme (1959) published his test-by-transplantation establishing unequivocal operational criteria for mouse mammary "preneoplasias". Foulds, using mouse models in his 1959 treatise on neoplastic progression, suggested a sequential acquisition of biological traits with linear progression. Wellings (1974), using the HAN model, classified human breast atypias identifying a linear histological continuum from atypia to cancer. Page extended these studies by correlating demographics with

histopathology. In retrospect, mouse models were limited to virus-induced tumors initiated by a limited number of oncogenes.
Genetic engineered mice have provided precancerous breast lesions initiated by oncogenes more directly related to human breast cancers. The test-by-transplantation has given operational proof of their biological potential. Transplanted mammary intraepithelial neoplastic outgrowths (MINO) mimic human DCIS when initiated by the same oncogene. MINO are morphologically, biologically and molecularly heterogeneous clonal growths. Upon serial transplantion, each clone reproduces its biological patterns. The major molecular and genetic changes occur during transitions from normal to MIN. Only minor changes occur during malignant transformation. This suggests pre-progammed cancer progenitor cells already exist in precancers and supports a parallel, rather than sequentual, model of neoplastic progression. A pre-programmed, parallel model has profound implications for the prevention and treatment of breast cancer.

GS1.4
GENOMIC STRATEGIES FOR PERSONALIZED CANCER TREATMENT

Nevins J. R.[1,2], Potti A.[1,2], Harpole D.[2], Ginsburg G.S.[1,2], Febbo P.[1,2], Bild A.H.[1,2], Dressman H.K.[1,2], and Lancaster J.M.[3]
[1]*Duke Institute for Genome Sciences and Policy,* [2]*Duke University Medical Center, Durham, NC, USA;* [3]*H. Lee Moffitt Cancer Institute, University of South Florida, Tampa, FL, USA*

The ability to tailor cancer therapy to characteristics of the individual patient is key in achieving a successful outcome. We have made use of genomic data to develop a capacity to predict risk for individual patients and then combine this with a capacity to identify potential therapeutic options. A series of gene expression signatures have been developed that have the capacity to predict sensitivity to both pathway-targeted agents as well as cytotoxic chemotherapeutics. Expression signatures were developed that reliably reflect the activation status of several oncogenic pathways. Predictions of pathway deregulation in cancer cell lines are shown to coincide with sensitivity to therapeutic agents that target components of the pathway, underscoring the potential for such pathway prediction to guide the use of targeted therapeutics. In parallel, we have also made use of in vitro drug response data generated with the NCI-60 panel of cancer cell lines, coupled with Affymetrix gene expression data, to develop genomic predictors of response and resistance for a series of commonly used chemotherapeutic drugs. Importantly, many of these predictors were then also validated with chemotherapeutic response data from patient samples, demonstrating a capacity to identify those patients most likely to respond to a given drug. When evaluated in several large collections of human cancers, these gene expression signatures of drug response identified patterns of predicted sensitivity suggesting their potential for guiding the use of novel combinations of these agents. Finally, we have combined the chemotherapy predictive signatures with the signatures of oncogenic pathway deregulation to identify therapeutic strategies that make use of all available drugs, matched to the characteristics of the individual patient. We suggest that this approach provides a strategy towards the development of personalized treatment options for the individual patient.

GS1.5
THE POTENTIAL OF COMBINED GENOMIC/ PROTEOMIC PROFILING TO INDIVIDUALISE BREAST CANCER THERAPY

Leyland-Jones B., Dobrolecki L., Hickey R., Smith I., Catzavelos C., Li Z., Provencher C., Dowsett M., Ponton A. and Abramovitz M.

The ability to use molecular profiling to rapidly characterize each breast tumour with respect to prognosis and treatment outcome is a critical step in tailoring the best possible treatment regimen to the individual patient. A number of microarray studies, using RNA prepared from frozen tumour samples, have uncovered sets of genes that have potential as prognostic and predictive signatures in breast cancer. Major drawbacks to validating these signature genes are that the majority of available tumour tissue samples exist as either formalin-fixed paraffin-embedded (FFPE) tumour blocks, eliminating, at this time, reproducible microarray studies. We will present our initial results with the Illumina DASL molecular profiling platform, that has been specifically designed for use with degraded RNAs such as those derived from FFPE tumor samples, demonstrating a remarkably robust correlation between receptor status, as measured by IHC and FISH, and receptor intensity, as measured in the DASL assay.
However, it has to be remembered that the key proteins driving oncogenesis can undergo post-

translational modifications. if we are ever to move individualization of therapy into the practical world of blood-based assays, serum proteomics becomes critical. Proteomic platforms, including tissue micro-arrays (TMA) and protein chip arrays, in conjunction with surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF/MS), have been the technologies most widely applied to the characterization of tumours and, most especially, serum which is routinely collected from breast cancer patients. We have conducted an initial proteomic analysis using SELDI on serum samples derived from IMPACT, a double-blind, randomized, neoadjuvant comparison of anastrozole with tamoxifen, alone or combined in postmenopausal patients with steroid receptor-positive primary breast cancer. We have derived a unique proteomic profile that appears to identify responding versus non-responding tamoxifen-treated patients: follow-up data on this study will be presented.

These are simply first generation platforms showing the application of reproducible genomic and proteomic platforms to routinely stored paraffin and serum. However, we believe this to be the future direction of individualized breast cancer therapy.

OP1 - ORAL PRESENTATIONS 1

OP1.1
THE SIX1 HOMEOPROTEIN IS A TRANSFORMING ONCOGENE THAT CONTRIBUTES TO MULTIPLE STAGES OF BREAST TUMORIGENESIS

Ford H.[1], McCoy E.[1], Christensen K.[1], Coletta R.[1], Micalizzi D.[1], Jedlicka P.[1] and Chodosh L.[2]
[1]UCDHSC , Aurora, CO, USA; [2]University of Pennsylvania, Philadelphia, PA, USA

Development and cancer share many properties. During development, genes are activated to stimulate cell proliferation and migration, and to regulate cell survival and cell-cell interactions. These genes are often downregulated once organ development is complete. During tumorigenesis, the same genes are often re-activated, stimulating the same properties out of context. The homeoprotein and transcription factor, Six1, exhibits such properties. Six1 is expressed in the mammary gland during embryonic development and early puberty, is lost in the differentiated mammary gland, but is re-expressed in breast cancer. Its overexpression, in part through gene amplification, is observed in 50% of primary breast cancers, suggesting that it may be involved in the etiology of the disease. Indeed, forced expression of Six1 in normal mammary epithelial cells can induce an epithelial-to-mesenchymal transition as well as transformation, leading to highly invasive ductal carcinomas in a nude mouse model. Furthermore, overexpression of Six1 in the mammary gland using an inducible transgenic mouse model leads to mammary epithelial hyperplasia, and in preliminary experiments, invasive ductal adenocarcinoma. Together, these data indicate that inappropriate expression of Six1 can contribute to the initiation of cancer. Interestingly, overexpression of Six1 is observed in an even larger percentage of metastatic breast tumors (90%), and its expression in breast cancer is significantly associated with the presence of lymph node metastasis ($p<0.05$). Indeed, overexpression of Six1 in tumorigenic, but nonmetastatic, MCF7 cells induces metastasis to lymph nodes and bone when the Six1-overexpressing cells are injected orthotopically into the mammary gland. Taken together, these data indicate that Six1 does not only initiate breast tumorigenesis, but can also promote tumor metastasis. As Six1 is involved in both proliferation and migration of progenitor cells during development, we hypothesize that its expression in adult tissues reinstates a developmental program that influences multiple pro-tumorigenic processes.

OP1.2
THE ROLE OF ZNF217, A GENE AMPLIFIED IN BREAST CANCER, DURING NEOPLASTIC PROGRESSION

Littlepage L.[1], Yaswen P.[2] and Werb Z.[1]
[1]University of California, San Francisco, San Francisco, CA, USA; [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA

The 20q13 region of the human genome is highly amplified in 20-30% of early stage human breast cancers, and this amplification correlates with poor prognosis. Work from several groups identified the human ZNF217 gene as one of two candidate oncogenes in 20q13.2 that correlates with breast cancer and gastric adenocarcinomas. ZNF217, a putative transcription factor, is a component of the corepressor of transcription associated with the human histone deacetylase complex (CoREST-HDAC), as well as in a complex with the transcriptional co-repressor C-terminal binding

protein (CtBP), and overexpression of ZNF217 in human mammary epithelial cell lines leads to immortalization of the cells. If ZNF217 is indeed a target oncogene in 20q13.2 driving breast cancer progression, then ZNF217 is a good candidate for targeted drug development. To investigate the effect of ZNF217 on neoplastic progression in epithelial cells, we cloned mouse Znf217 by PCR into a retroviral vector and infected mouse epithelial SCp2 cells with vector or mouse Znf217. Using an antibody raised against human ZNF217 that we found cross-reacts with mouse Znf217, we found that this construct expresses the Znf217 protein when induced, as determined by western analysis. Cells overexpressing Znf217 had altered cell and nuclear morphology and showed increased motility in scratch assays.. In addition, we characterized the role of Znf217 as a putative transcription factor. Using Gal4-fusion Znf217 constructs in transcription assays, we found that ZNF217 is a strong transcriptional repressor. Our data suggest that repression of a transcriptional target by ZNF217 may lead to increased motility of epithelial cells in culture. Further characterization of downstream targets and expression patterns will elucidate the pathways affected by ZNF217 in breast cancer progression.

OP1.3
HOW RECEPTORS FOR ECM AND SOLUBLE FACTORS ARE INTEGRATED TO REGULATE TISSUE-SPECIFIC GENE EXPRESSION IN THE MAMMARY GLAND

Katz E., Akhtar N., Cheung J., Lambert E., Lowe E., Marlow R., Schatzmann F., Naylor M. and Streuli C.
University of Manchester, Manchester, UK

Adhesive interactions with the extracellular matrix (ECM) regulate many aspects of normal mammary epithelial cell (MEC) behaviour, including survival, proliferation and migration. ECM is also central for mammary differentiation. Endocrine hormones, such as prolactin, regulate transcription of milk protein genes (e.g. β-casein) in a developmental context in vivo. The prolactin/Stat5 signalling axis is reliant on MEC being in the correct spatial location and in contact with appropriate ECM. Our laboratory has used the mammary gland system as a paradigm to dissect the molecular basis of signal integration by ECM and soluble factors. Previously, we have demonstrated that the ECM protein laminin and its integrin receptor are critical for β-casein expression. We now demonstrate key roles for the β1-subunit of integrin, an intermediate of integrin function, integrin-linked kinase (ILK), and the small GTPase, Rac1, for signal integration between the integrin and prolactin receptors.

a) By conditionally deleting the β1-integrin gene from luminal epithelia in vivo and in cultured primary MEC, we have demonstrated that integrins are essential to maintain functional differentiation in glandular epithelium. Lactation was severely defective when β1-integrin was deleted from mammary epithelium in vivo, and the β1-integrin null MEC did not differentiate in response to prolactin stimulation, due to defective Stat5 activation.

b) To understand how integrins influence prolactin signalling and differentiation, we have determined the role of both proximal and distal proteins. Conditional ablation of ILK demonstrated its requirement for mammary gland development and differentiation in vivo, as well as β-casein expression and prolactin/Stat5 signalling in cultured MEC.

c) We have demonstrated that dominant negative Rac1 inhibited prolactin signalling. Conversely, a constitutively activate Rac1 rescued differentiation in both β1-integrin-null and ILK-null cells. This identifies a specific enzyme, Rac1, as a link between integrin and prolactin receptors in coordinating cellular differentiation.

OP1.4
NRDP1 INHIBITS THE GROWTH AND MOTILITY OF BREAST CANCER CELLS BY INHIBITION OF ERBB3-MEDIATED SIGNAL TRANSDUCTION

Carraway, III K.[1], Yen L.[1], Cao Z.[1], Ingalla E.[1], Liu S.[2], Young L.[3], Gregg J.[2], Cardiff R.[3], Borowsky A.[3] and Sweeney C.[1]
[1]UC Davis Cancer Center, Sacramento, CA, USA; [2]UC Davis, Sacramento, CA, USA; [3]UC Davis, Davis, CA, USA

Dysregulation of ErbB tyrosine kinases is thought to promote mammary tumor progression by stimulating tumor cell growth and invasion. Overexpression and aberrant activation of ErbB2/HER2 confers aggressive and malignant characteristics to breast cancer cells, and patients displaying ErbB2-amplified breast cancer face a worsened patient prognosis. Of significance is the

strong propensity of ErbB2 to heterodimerize with and activate the ErbB3 receptor. ErbB2 and ErbB3 have been shown to synergize in promoting growth factor induced proliferation, transformation, and invasiveness. Recent studies have established a strong link between the overexpression and activation of ErbB2 and ErbB3 in breast tumor cell lines and in patient samples. Our previous studies have shown that the Nrdp1 E3 ubiquitin ligase specifically suppresses cellular ErbB3 levels by marking the receptor for proteolytic degradation. Our current studies reveal that overexpression of Nrdp1 in human breast cancer cells results in downregulation of ErbB3 levels, with inhibition of cell growth, motility, and attenuation of signal transduction pathways. In contrast, overexpression of a dominant negative form of Nrdp1 in human breast cancer cells results in enhanced cellular proliferation and augmentation of downstream signaling pathways. Additionally, Nrdp1 expression was found to be inversely correlated with ErbB3 levels in primary human breast cancer tissue and in a mouse model of ErbB2 mammary tumorigenesis. Our observations suggest that downregulation of ErbB3 by Nrdp1 suppresses the growth and motility of tumor cells and raise the prospect that Nrdp1 may ultimately be useful as a therapeutic agent in ErbB2- or ErbB3-positive breast cancer. Additionally, the studies could lead to the development of Nrdp1 loss as a marker of invasive breast tumors.

OP1.5
POLARITY GENES ARE CRITICAL REGULATORS OF ONCOGENE-INDUCED TRANSFORMATION OF POLARIZED EPITHELIAL CELLS

Muthuswamy S.[1], Aranda V.[1], Nolan M.[2] and Haire T.[2]
[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

The loss of tissue organization is a defining feature of all carcinomas, cancers of epithelial origin that make-up the majority of human malignancies. Luminal epithelial cells in normal glands are organized as single layers with apical-basal polarity. The polarized organization of epithelia is lost early during neoplastic transformation, by mechanisms that are not well understood. We identify a mechanism used by oncogenes to disrupt epithelial organization. Activation of ErbB2, an oncogenic receptor tyrosine kinase, initiated disruption of epithelial architecture at the apical-lateral border that progressed to a loss of apical polarity. ErbB2 directly recruited and regulated Par6-aPKC, a polarity complex, which controls establishment of apical-lateral border. Interfering with the ability of ErbB2 to recruit Par6-aPKC blocked ErbB2-induced disruption of epithelial organization, but not cell proliferation in an organotypic culture system. Thus, ErbB2 disrupts epithelial organization through pathways that are independent of its ability to induce cell proliferation.
In the absence of ErbB2-Par6-aPKC interaction, ErbB2-induced proliferation resulted in increased apoptosis, suggesting that oncogene-induced proliferation in the absence of a signal to disrupt polarity induces apoptosis. In conclusion, we have defined a novel mechanism, that involves polarity genes, to regulate early stages of oncogene-induced transformation of organized epithelial cells.

OP1.6
STEROID RECEPTOR COACTIVATOR 2 IS CRITICAL FOR PROGESTERONE-DEPENDENT MAMMARY MORPHOGENESIS IN THE MOUSE

Lydon J.
Baylor College of Medicine, Houston, TX, USA

Although the essential involvement of the progesterone receptor (PR) in female reproduction and mammary morphogenesis is firmly established, the coregulators preferentially enlisted by PR to mediate its physiological effects have yet to be fully delineated. To further dissect the roles of members of the steroid receptor coactivator (SRC)/p160 family in PR mediated mammary morphogenesis in vivo, state-of-the-art cre-loxP engineering strategies were employed to generate a mouse model (PRCre/+SRC-2flox/flox) in which SRC-2 function was abrogated only in cell-lineages that express the PR. Apart from an infertility defect, the PRCre/+SRC-2flox/flox female exhibited a severe impairment in P-induced ductal side-branching and alveologenesis in the mammary gland. Like the progesterone receptor knockout (PRKO) mammary phenotype, the defect in PRCre/+SRC-2flox/flox mammary morphogenesis was underscored by a block in mammary epithelial proliferation with an accompanying decrease in expression of a select subset of known mammary progestin targets such as Wnt-4, osteoprotegrin ligand, amphiregulin, and calcitonin. Importantly, the expression levels of SRC-1 and SRC-3 in the PRCre/+SRC-2flox/flox mammary gland were not

affected by the absence of SRC-2 thereby strengthening the conclusion that the PRCre/+SRC-2flox/flox mammary phenotype is solely due to the abrogation of SRC-2 function. That SRC-2 is selectively appropriated by PR in a subset of transcriptional programs that lead to significant cellular proliferation in the mammary gland promises to provide a broader conceptual framework for further understanding abnormal progestin responses in this target tissue which can often lead to aberrant growth and cancer.

OP1.7
HER-2 IS THE PRIMARY DRIVER OF ALTERED CELL SIGNALING IN EGF-INDEPENDENT BREAST CANCER CELLS

Ethier S.
Karmanos Cancer Institute, Wayne State University, School of Medicine, Pathology, Detroit, MI, USA

In previous work, we demonstrated an association between overexpression of p185-Her-2 that occurs in human breast cancer (HBC) cells with a HER-2 gene amplification, and the acquisition of EGF-independent growth and survival in vitro. More recently, we demonstrated that over expression of HER-2 in normal human mammary epithelial (HME) cells is not sufficient to confer the EGF-independence phenotype. However, HER-2 over expression can cooperate with other oncogenes and oncoproteins to mediate growth factor independence, which is associated with malignant and metastatic growth potential in vivo. In the present studies, we compared the activation of HER family members in HME cells that over express HER-2, but which retain their EGF-dependence, to their fully transformed counterparts that have acquired EGF-independence. Our results demonstrate that HER-2 over expressing but EGF-dependent HME cells activate HER-2 by well described mechanisms involving heterodimerization with either EGFR or HER-3, both of which require ligand for activation and signal transduction. By contrast, HER-2 over expressing but EGF-independent HME cells and breast cancer cells exhibit constitutive activation of HER-2 that is independent of exogenous ligands. Furthermore, experiments using a HER-2 specific kinase inhibitor that has no direct effects on EGFR activity demonstrated that EGFR tyrosine phosphorylation in these cells is completely dependent on the kinase activity of HER-2. This is in stark contrast to the situation in EGF-dependent cells, where ligand-dependent activation of EGFR is required for HER-2 tyrosine phosphorylation. Thus, the transition to EGF-independence that occurs in breast cancer cells with a HER-2 amplification is accompanied by a transformation in the signaling function of p185Her-2. In EGF-dependent cells, HER-2 acts as a co-receptor that requires ligand-mediated interaction with other HER family members. However, in EGF-independent cells, HER-2 is a dominant signaling molecule that is constitutively active and mediates EGFR-tyrosine phosphorylation in a ligand-independent manner.

OP1.8
ELF5 AND MAMMARY GLAND DEVELOPMENT

Ormandy C.[1], Oakes S.[1], Naylor M.[1], Kazlaukas M.[1], Blazek K.[1], Chodosh L.[2] and Pfeffer P.[3]
[1]Garvan Institute of Medical Research, Sydney, Australia; [2]Abramson Institute, Philadelphia, PA, USA; [3]AgResearch, Ruakura, New Zealand

The genetic regulatory network specifying mammary gland development in response to prolactin and progesterone, the major pregnancy hormones, remains largely unknown. To discover this program we compared transcript profiles derived from prolactin receptor knockout and wild type epithelium during early pregnancy. Further comparison with profiles from prolactin treated Scp2 cells revealed that induction of expression of the ets transcription factor Elf5 failed in the absence of prolactin signalling. Retroviral reintroduction of Elf5 expression in prolactin receptor null mammary epithelium rescued the failure of lobuloalveloar development during pregnancy that characterises prolactin receptor null glands. Knockout of Elf5 was embryonic lethal due to loss of the extraembryonic ectoderm. Tetraploid rescue of the placentation defect produced a viable female that failed to lactate. Transplantation of Elf5 knockout mammary epithelium allowed a detailed investigation of mammary development. Ductal morphogenesis was normal but lobuloalvelar development failed during early pregnancy, a similar defect to that produced by loss of the prolactin receptor. Knockdown of Elf5 in HC11 mammary epithelial cells showed that Elf5 was necessary for progress through all phases of the cell cycle. Subsequent differentiation in response to lactogenic hormones was also blocked. A transgenic mouse was constructed to allow mammary specific and tet inducible Elf5 expression, marked by EGFP. Forced expression of Elf5 in virgin animals from 5 weeks of age stopped ductal elongation due to precocious

epithelial differentiation within the terminal end bud. Alveoli formed throughout the gland and the nipple ductal sinuses distended with EGFP laden milk. Milk protein expression increased by 3 orders of magnitude. Thus Elf5 is a prolactin-induced transcription factor capable of substituting for signalling via the prolactin receptor and essential for the proliferation and differentiation of mammary epithelial cells. Inappropriate expression can cause premature cell differentiation. Elf5 may be a master regulator of mammary development during pregnancy.

GS2 – GENERAL SESSION 2: SIGNAL TRANSDUCTION

GS2.1
THE ERBB FAMILY OF RECEPTOR TYROSINE KINASES AND TUMOR CELL BIOLOGY

Hynes N.E., Masson R., Jacob S. and Kaeser P.
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

The ErbB receptors have essential roles in normal physiology and are involved in the development of numerous types of human tumors. Cancer development is a multistep process starting from a local benign hyperplasia and ending with an invasive tumor able to metastasize to distant organs. During this process cancer cells acquire properties that are necessary for the full malignant phenotype. ErbB receptors and their ligands have been shown to play roles in many of these processes, for example, in tumor cell motility, an important characteristic of metastatic cells. We recently identified Memo (mediator of ErbB2-driven motility), a novel ErbB2 PY binding protein that is essential for receptor induced cell motility. Memo is conserved through evolution and Memo homologues are found in all branches of life. We have generated knock-out mice for Memo and have shown that homozygous mutant mice die at midgestation (E13.5). Memo -/embryos shows defects in placental vascularization and heart development. To address whether Memo has a role in metastatic cancer development, we used the highly metastatic mouse mammary tumor cell line 4T1. A shRNA vector designed to stably down-regulate Memo expression was introduced into 4T1 cells and clones showing moderate to strong decrease in Memo level were selected. The in vitro migratory ability of Memo knock-down cells in response to serum was decreased in comparison to control cells. Importantly, we observed a lower rate of lung metastasis from the primary mammary tumor that correlated with Memo expression level in the knock-down cells, suggesting that Memo has an important role in the metastatic process.

GS2.3
TYROSINE PHOSPHATASE EPSILON AS A SUPPORTER OF NEU-INDUCED MAMMARY TUMORIGENESIS

Elson A., Berman-Golan D. and Gil-Henn H.
The Weizmann Institute of Science, Rehovot, Israel

Despite the well-established role of aberrant tyrosine phosphorylation in promoting malignancy, relatively few tyrosine phosphatases have been studied in this context. We present genetic and biochemical evidence that the receptor-type tyrosine phosphatase epsilon (RPTPε) supports transformation of mammary epithelial cells induced *in vivo* by Neu. Cells from mammary tumors induced by activated Neu in mice genetically lacking RPTPε appear less transformed morphologically and proliferate slower *in vitro* and *in vivo*. We show that RPTPe dephosphorylates and activates c-Src in mammary tumor cells and that the above cellular phenotypes are caused by reduced c-Src activity secondary to lack of RPTPe. We also show that activation of c-Src by RPTPe is regulated by Neu itself: Neu induces phosphorylation of RPTPε exclusively at its C-terminal Y695, and this phosphorylation is required for activation of c-Src by RPTPε. Neu-induced phosphorylation appears to direct existing RPTPe activity towards c-Src without increasing phosphatase activity towards other, non-Src substrates of RPTPe. RPTPε is subject to strong auto- and trans-dephosphorylation activities, suggesting this as a mechanism that limits activation of c-Src downstream of Neu to the immediate vicinity of Neu. We conclude that a Neu-RPTPε-Src signaling pathway exists in mammary tumor cells, in which phosphorylation of RPTPε by Neu directs the phosphatase to activate c-Src. The data also suggest that reversible phosphorylation of RPTPε at Y695 is a general physiological "molecular switch" that affects the substrate specificity of this phosphatase.

GS2.5
MODELING CANCER IN THREE DIMENSIONS IN VITRO

Brugge J. S., Collins N., Irie H.Y., Gao S., Cheng F., Overholzer M., Carroll D., Knowlton M., Mailleux A., Schmelzle T.
Department of Cell Biology, Harvard Medical School, Boston, MA, USA

The proliferation, survival and branching of mammary epithelial cells is highly influenced by interactions of the epithelial cells with other cells, with their surrounding extracellular matrix, and with hormones and other regulatory factors in their microenvironment. Oncogenes, tumor suppressor genes, and factors in the microenvironment allow cells to escape regulatory controls imposed on cells in their natural tissue environment, thus leading to progressive changes in the organization of cells, their proliferative capacity, their sensitivity to stress-induced death and their ability to escape from their natural environment. We have utilized an *in vitro* 3-dimensional model in which epithelial cells are able to organize into hollow, spherical structures that resemble glandular acini in order to reconstruct oncogene-induced events that are involved in the initiation and progression of cancer, to identify cellular pathways that mediate these events and to develop methods to determine the activity state of these pathways in human tumors. We have previously shown that filling of the luminal space of the acinar-like structures in vitro requires both anti-apoptotic activities as well as proliferative activities. My presentation will highlight the use of 3D models to probe the biological activities of candidate oncogenes that are associated with human breast tumors and to identify proteins that are required for phenotypic alterations that are critical for tumor cell proliferation and survival.