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GS3 – GENERAL SESSION 3: RECEPTORS AND HORMONES

GS3.1
LINKING THERAPY TRIALS IN MODEL SYSTEMS WITH CLINICAL TRIALS

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The US-National Cancer Institute's (NCI) six-year investment in a collaborative program of cancer modeling in laboratory mice provides a wealth of new strains whose biology increasingly are excellent simulations of the corresponding human cancer. The success of the NCI-Mouse Models of Human Cancers Consortium (NCI-MMHCC) in deriving new cancer models prompted the program to engage in numerous pilot projects to define how and when to use genetically engineered mouse models in development and testing of cancer interventions. In collaboration with the NCI, the Consortium is delineating the requirements for an experimental therapeutics infrastructure that will permit researchers to apply the emerging variety of models systems and approaches – *in vitro, in vivo,* and *in silico* – iteratively and integratively to inform the conduct of early clinical trials.

In parallel with the on-going projects to define how and when to use which kind of model, the Consortium works with the NCI Center for Bioinformatics (NCI CB) to develop the informatics infrastructure to support preclinical therapy testing and integration of those data with data from early clinical trials. The NCI-CB has a project, caBIG™ (cancer Biomedical Informatics Grid), that is evolving to connect individuals and institutions to enable the sharing of data and tools, creating a World Wide Web of cancer research. The goal is to speed the delivery of innovative approaches for the prevention and treatment of cancer. One aspect of the Grid is informatics support of clinical trials; another is information about the biology, histopathology, and gene expression analysis of mouse cancer models, and results of testing drugs in them. The Consortium and NCI CB developed the latter informational databases collaboratively; now they are working together to generate further enhancements to enable full support of experimental therapeutics.

GS3.4
A SYSTEM LEVEL APPROACH TO ONCOGENIC SIGNALING BY HER2 AND EGFR IN BREAST CANCER

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Growth factors and their transmembrane receptors contribute to all steps of tumor progression; from the initial phase of clonal expansion, through angiogenesis to metastasis. Hence, the information relay system involved in growth factor signaling provides potential site of signal interception and tumor inhibition. A relevant example comprises the epidermal growth factor (EGF) and the respective receptor tyrosine kinase, ErbB-1/EGFR, which belong to a prototype signaling module that drives mammary development. The extended module includes another autonomous receptor, ErbB-4, and two non-autonomous receptors, namely: a ligand-less oncogenic receptor, HER2/ErbB-2, and a kinase-dead receptor (ErbB-3). This signaling module is richly involved in human cancer and already serves as a target for several cancer drugs. Due to inherent complexity and a large amount of experimental data, we propose a systems biology approach to coping with ErbB signaling. EGF - to - ErbB signaling is envisioned as a bow-tie configured, evolvable network, sharing modularity, redundancy and control circuits with robust biological and engineered systems. This presentation will concentrate on system controls, a plethora of negative feedback loops, which include E3 ubiquitin ligases, receptor endocytosis and newly transcribed genes. Because network fragility is an inevitable tradeoff of robustness, system level understanding is expected to identify therapeutic opportunities for targeting

aberrant activation of the network in human pathologies.

GS3.5
STRUCTURAL BASIS FOR EGF/ERBB RECEPTOR SIGNALING AND ERBB-TARGETED CANCER THERAPIES

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The ErbB family of receptor tyrosine kinases in humans comprises the Epidermal Growth Factor Receptor (EGFR, ErbB1, HER1), ErbB2 (HER2, Neu), ErbB3 (HER3), and ErbB4 (HER4). These receptors consist of an extracellular ligand-binding region, a single transmembrane spanning region, and a cytoplasmic tyrosine kinase. Ligand binding to the extracellular regions of these receptors leads to receptor dimerization, activation of the tyrosine kinase, and initiation of an intracellular signaling cascade. Each of these receptors promotes cell growth and differentiation at multiple stages of embryonic development, and inappropriate activation of these receptors has been shown to contribute to the cause and severity of several human cancers. Inhibiting activated ErbB receptors has proven useful for treating human cancer, and two monoclonal antibodies against ErbBs, Erbitux and Herceptin, and two small-molecule kinase inhibitors, Tarceva and Iressa, have been approved for treatment of specific cancers. Recently my lab, along with several others, has determined X-ray crystal structures of the extracellular regions of each of the ErbB receptors both with and without ligand. These structures have revealed a "closed" structure for ErbB1, ErbB3, and ErbB4 in the absence of ligand that undergoes a large conformational change when ligand binds. This conformational change promotes dimerization and activation of the tyrosine kinase. Curiously, the ErbB2 structure revealed a constitutively "open" or active-like structure in the absence of ligand, which explains the absence of an ErbB2/HER2 ligand, the ability of ErbB2/HER2 to partner with each of the other ErbBs regardless of stimulating ligand, and may explain its greater oncogenic potential. We and a group at Genentech have also determined the crystal structures of ErbB2 bound to two different therapeutic monoclonal antibodies, Herceptin and Omnitarg, and these structures help explain the different biochemical and therapeutic properties of these antibodies.

PL2 – PLENARY 2

PL2
MECHANISMS OF RESISTANCE TO ESTROGEN DEPRIVATION AND STRATEGIES TO OVERCOME THEM

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Estrogen deprivation therapy has been an important component of breast cancer treatment dating back more than 100 years. Estrogen deprivation therapy in premenopausal women involves surgical or medical ovarian ablation to reduce the level of circulating estrogen to postmenopausal levels. Estrogen deprivation therapy in postmenopausal women involves the use of aromatase inhibitors. In contrast to tamoxifen, very little is known about the mechanism for resistance to estrogen deprivation therapy in either premenopausal or postmenopausal women. Clinical data do suggest that the mechanisms for resistance to aromatase inhibitors are different than that of tamoxifen since aromatase inhibitors are known to be effective when tamoxifen resistance develops. Clinical data also suggest that aromatase inhibitors may be more effective than tamoxifen in tumors overexpressing HER2-*neu* or in those with absent progesterone receptors which may also be a marker of excessive growth factor receptor activity. *In vitro* and *in vivo* preclinical models have explored possible mechanisms of resistance to estrogen deprivation therapy. In tumors which express ER but do not overexpress HER2 preclinical data suggests that the level of ER is upregulated and tumors become exquisitely sensitive to minute amounts of residual estrogen in the environment. This super sensitivity to estrogen may involve crosstalk with growth factor receptor pathways. Although aromatase inhibitors are effective initially in patients with HER2 overexpressing tumors in the clinic as well as in preclinical models, resistance develops rapidly. Resistant tumors are characterized by loss of ER rather than increased ER expression and by upregulation of growth factor signaling pathways. Preclinical data suggest that combinations of estrogen deprivation therapy with growth factor receptor inhibitors can be very effective in preventing the development of resistance and eradicating human breast cancers growing as xenografts in nude mice. There strategies are now being testing in clinical trials.

PL3 – PLENARY 3

PL3
THE TUMOR MICROENVIRONMENT: HOST CELL-TUMOR INTERACTIONS THAT DEFINE THE INVASION SIGNATURE

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The recent convergence of technologies for expression profiling and intravital imaging has revealed the identities of the genes involved in the survival, adjuvant-resistance and chemotaxis of invasive cancer cells inside living tumors. These genes fall into well defined pathways and are coordinately regulated in invasive tumor cells. This pattern is called the invasion signature. The invasion signature indicates that invasive cancer cells are a population that is neither proliferating nor apoptotic but highly chemotactic to macrophage-secreted EGF. Of particular relevance to the migratory behavior of invasive cancer cells is the finding that the genes coding for pathways that regulate chemotaxis to EGF, i.e., the cofilin, Mena and Arp2/3 pathways, are coordinately up-regulated. This latter result is particularly relevant to the contribution of the tumor microenvironment to metastasis because multiphoton imaging of living tumors in mice has shown that chemotaxis to blood vessels is involved in the escape of cancer cells from primary mammary tumors. Key genes in the invasion signature have been studied for their ability to alter metastatic outcome and these results confirm the importance of the invasion signature in predicting metastasis. Finally, the invasion signature provides several new target opportunities for preventing metastasis and testing of these will be discussed.

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ORAL PRESENTATIONS 2

OP2.1
EFFICIENT GENERATION OF SPONTANEOUS HUMAN BREAST CANCERS IN MICE WITH DEFINED GENETIC ELEMENTS

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The development of cancer is a complex pathobiological process that is influenced profoundly by the specific combination of acquired mutations, species-specific differences in signaling circuits, the cell type and tissue microenvironment. While hundreds of cancer-relevant genetic and epigenetic events have been identified across many different human tumor types, we possess an elemental understanding of the functional significance of these alterations, their biological actions in the transformation process, and the physiological and clinical context in which these lesions operate to influence tumor development and response to therapy. Here, we sought to generate an accurate in vivo model of human breast cancer in mice by employing a unique tissue recombinant system comprised of human stroma and breast epithelial cells engineered with defined genetic elements. In this human breast cancer in mice (HIM) model, spontaneous invasive breast carcinomas develop as early as 5 weeks post-implantation of primary breast epithelial cells engineered with a series of dominant oncogenes such as HER2/NEU/erbB2, Kras and/or PIK3CA, and with dual inactivation of the RB and p53 tumor suppressor pathways. The histological presentation of these HIM tumors closely resembled either invasive, low-grade human ductal adenocarcinomas or highly malignant carcinomas encounteed in human patients. In striking contrast to the transformation of cultured human mammary epithelial cells (HMECs), these primary HIM tumors exhibit spontaneous robust activation of endogenous telomerase in the absence of hTERT transduction. Finally, consistent with the critical role of the microenvironment in human breast cancer

pathobiology, HIM tumor development is shown to be highly dependent on specialized stromal fibroblasts, as tumors rarely develop in the absence of this microenvironment. The HIM model provides a defined genetic and biological system in which to validate cancer gene candidates, determine their biological roles in transformation, and test targeted therapies directed against those genetic lesions resident in human breast cancer.

OP2.2
ROLE OF THE HLH PROTEIN ID1 IN MAMMARY TUMORIGENESIS

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Significant evidence suggests that elevated expression of the HLH transcriptional regulator Id1 is found in a majority of advanced breast cancers and is predictive of poor patient outcome. However, the contribution of Id1 to breast cancer etiology is contentious and has not been experimentally modeled in vivo. Using a conditional transgenic model we show that overexpression of Id1 alone in the mammary epithelium is not sufficient for neoplasia. To screen for pathways whose disruption cooperated with Id1 in tumorigenesis, we developed a conditional orthotopic mammary transplant model and identified activated h-Ras (RasV12) as a cooperating factor. While the majority of transplants of cells expressing RasV12 alone did not develop tumors, those expressing both Id1 and RasV12 formed high grade metastatic adenocarcinomas. These tumors expressed markers of mixed epithelial lineages, suggesting transformation of a progenitor cell. We then tested the requirement for Id1 in tumor maintenance. Following Id1 de-induction by doxycycline administration, all tumors ceased growth, with ~50% undergoing regression. To investigate the mechanism of regression, cells cultured from these tumors were treated with doxycycline in vitro. Within 5 days cultures underwent cellular senescence, characterized by flattened morphology and senescence-associated b-galactosidase (SA-bGal) activity. To test whether senescence also played a role in vivo, we stained for SA-bGal in the mammary glands of transplant recipients. RasV12-expressing cells at the site of injection were positive for SA-bGal while those co-expressing RasV12 and Id1 were not. These data identify Id1 as a mammary oncogene and suggest that Id1 cooperates with Ras pathway activation by circumventing the tumor suppressor mechanism of premature senescence.

OP2.3
ROLE OF PTP1B IN BREAST CANCER DEVELOPMENT

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Because most oncogenes originally described were tyrosine kinases, PTPs were first postulated to be tumor suppressor genes. The role of PTP1B is unclear: PTP1B protein levels have been found to be increased in an important proportion of breast and ovarian cancers, but decreased in the esophageal cancers. Our published work and ongoing studies are revealing the oncogenic properties of PTP1B. We and others have demonstrated that PTP1B-deficient fibroblasts display increased IGF-I receptor, PDGRF and EGFR tyrosine phosphorylation and associated PI3K-mediated signaling, but activation of their Ras/MAPK-mediated pathways is significantly impaired. Examining this phenomenon further, we showed that loss of PTP1B resulted in decreased Ras activity and that this event occurred through increased p120RasGAP expression and augmented p62Dok phosphorylation. Thus, PTP1B could potentially play a prooncogenic role by enhancing Ras signaling. To examine this hypothesis, we have recently begun evaluating the consequence of removing PTP1B in the MMTV-ErbB2 model of breast cancer developed. Our very recent data indicate that PTP1B+/+ and PTP1B+/- ErbB2 transgenic (TG) mice present a defect in lactation and postnatal death of their progeny occurs due to tumor initiation. In contrast, PTP1B deficient -/- ErbB2 transgenic (TG) mice have normal pups and they are able to nurse their young to a normal weight. Even more striking, PTP1B WT and heterozygote ErbB2 transgenic animals have detectable tumors at 4 months of age, but knockout PTP1B MMTV-ErbB2 animals have yet to develop tumors at the same age. Although quite preliminary these findings are remarkable and suggest that indeed PTP1B could be an important factor in the process of breast cancer initiation and development. Our main objective remains understanding PTP1B function in order to acquire new means to influence cell and body metabolism for the treatment of human diseases, particularly cancer.

OP2.4
MICROENVIRONMENT OF THE INVOLUTING MAMMARY GLAND ACTIVATES TUMOR METASTASIS

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First full term pregnancy in older women increases breast cancer risk and increases the odds of death from metastasis. Studies find that the poorer survival of pregnancy-associated breast cancer is independent of known prognostic factors and of delayed diagnosis. Because the tumor microenvironment is rate-limiting for metastasis, we tested the hypothesis that physiologic mammary gland regression after pregnancy promotes dissemination of pre-existing but previously quiescent tumor cells. To this end, ECM was isolated from mammary glands of rats that were nulliparous or whose glands were undergoing weaning induced involution. Biochemical characterization of these matrices identified attributes of wound healing stroma only in involution-matrix, including elevated MMP activity, bioactive fragments of fibronectin, increased cytokine levels and increased fibrillar collagen. Using these matrices as substratum for hMCC, nulliparous-matrix suppressed, whereas involution-matrix promoted tumor cell invasiveness in vitro. To evaluate the effects of these matrices on metastasis of MDA-231 cells in vivo, 48 nude mice were injected in the inguinal MFP with tumor cells that were premixed with Matrigel, nulliparous or involution-mammary matrix. Metastases to lung, liver and kidney were significantly increased in mice from the involution-matrix group. Metastasis correlated with an increase in tumor VEGF expression and increased angiogenesis. These data support the hypothesis that during mammary gland involution, the tissue microenvironment becomes promotional for tumor cell invasion and angiogenesis, thus providing a plausible mechanism to explain the high rate of metastases that occur with pregnancy-associated breast cancer. This work identifies the window of mammary gland involution as a promising target for prevention strategies.

GS4 - GENERAL SESSION 4: TUMOR MICRO-ENVIRONMENT AND CANCER STEM CELLS

GS4.2
THE TUMOR MICROENVIRONMENT EDUCATES MACROPHAGES TO PROMOTE TUMOR PROGRESSION AND METASTASIS

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Leukocytic cells populate the microenvironment of all solid tumors. Of these cells, macrophages are particularly abundant. Although originally these macrophages were considered to be detrimental to the tumor, most clinical studies have shown that their density is correlated with poor prognosis suggesting that they may be pro-tumorigenic. To discriminate between these two conflicting views, we used genetic means to deplete macrophages from a mouse model of breast cancer caused by the mammary epithelial restricted expression of the Polyoma Middle T oncoprotein. This depletion did not affect tumor incidence or initial growth rates but it did impede the progression to malignancy and almost completely ablated pulmonary metastasis when compared with the wild type macrophage-replete mice. Restoration of macrophages to the tumors of mutant mice restored these phenotypes to wild type levels and in wild type mice, premature recruitment of macrophages to the pre-malignant tumor accelerated their progression to malignancy and increased their metastatic rate. These data strongly suggest that macrophages are required for the full expression of the malignant potential of tumors.

Our studies to define the role of macrophages in promoting malignancy have used intravital imaging of fluorescently labeled cells together with a novel micro-capillary invasion assay. These experiments have shown that at the invasive front there is a paracrine interaction between EGF-synthesized by macrophages and CSF-1 by tumor cells that is required for tumor cell migration and intravasation into the circulation. In addition, we have shown that macrophages promote tumor angiogenesis particularly at the point of malignant transition. These functions together would enhance metastasis and therefore, they provide at least part of the explanation of why macrophage depletion inhibits tumor progression and metastasis. These data also suggest an explanation for the clinical association of increased macrophage density with poor prognosis in human breast cancers. Thus, these experimental and clinical data suggest that

inhibition of specific macrophages functions should have significant therapeutic benefit.

GS4.4
HEREDITARY BREAST CANCER: FROM PATHOLOGY TO TREATMENT AND BEYOND

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Over the last few years, it has become apparent that *BRCA1*-related breast cancers (B1BC) have a rather specific phenotype: infiltrating ductal breast cancers that show expression of "basal" proteins that are usually only found in basally-situated cells in the normal breast. This phenotype is negatively associated with expression of both estrogen receptor and HER2. The tumors are often high grade and show aneuploidy. It has been much harder to identify a specific phenotype for *BRCA2*-related breast cancers (B2BC). One of the most practical uses of the distinctive pathology of B1BC is to use it to improve the specificity of genetic testing, which is an expensive process. For example, among ER and HER2 negative breast cancers, CK5/6 positivity is strongly associated with the presence of a germ-line *BRCA1* mutation. Another approach is to use CGH; one study has identified a potential classifier of B1BC involving loss of chromosomes 3p and 5q and gain of 3q. As discussed above, identifying B2BC is difficult, but if testing for B12BC is to be done sequentially, then identifying B2BC first could be of use. Refining a robust B2BC CGH classifier that could distinguish B2BC from non-B12BC would be of considerable help. As both *BRCA1/2* are clearly implicated in DNA repair, and deficient DNA repair in a tumor may mean that errors introduced by chemotherapeutic agents cannot be repaired, and this could result in preferential activation of programmed cell death pathways in B12BC. For this reason, there is some reason to believe that B12BC, lacking efficient DNA repair, may be particularly susceptible to agents that act directly on DNA, e.g. platinum, mitomycin C. The real excitement comes, however, from the possibility that "triple negative" breast cancers (i.e. ER-, PR- and HER2-negative) may be susceptible to new biological therapies. The vascular phenotype of B1BC offers special opportunities for anti-angiogenic therapies.

GS4.5
THE ROLE OF NOTCH SIGNALING IN CELL FATE DECISIONS IN THE MAMMARY GLAND

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Mammary alveoli are composed of luminal (secretory) and basal (myoepithelial) cells, which are descendants of a common stem cell. This study addressed the role of RBP-J-dependent Notch signaling in the formation, maintenance and cellular composition of alveoli during pregnancy. For this purpose, the genes encoding RBP-J, the shared transcriptional mediator of Notch receptors, and Pofut1, a fucosyltransferase required for the activity of Notch receptors, were deleted in mammary progenitor cells in the mouse using Cre-mediated recombination. Loss of RBP-J and Pofut1 led to an accumulation of basal cell clusters characterized by the presence of cytokeratins K5 and K14 and SMA during pregnancy. Hormonal stimulation of mutant tissue induced the expression of the basal cell transcription factor p63 in luminal cells and excessive proliferation of basal cells. A transient enrichment of K6-positive luminal cells was observed upon hormonal treatment suggesting a temporary arrest at an immature stage prior to transdifferentiation and expansion as basal cells. Despite the extensive proliferation of RBP-J-null basal cells during pregnancy, hormonal withdrawal during involution resulted in complete remodeling and the restoration of normal tissue architecture. We propose that the Notch-RBP-J pathway regulates alveolar development during pregnancy by maintaining luminal cell fate and preventing uncontrolled basal cell proliferation.

GS4.6

"HEDGEHOG AND NOTCH SIGNALING REGULATES SELF-RENEWAL OF NORMAL AND MALIGNANT HUMAN MAMMARY STEM CELLS THROUGH REGULATION OF THE POLYCOMB GENE BMI-1"

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The epithelial components of the mammary gland arise from stem cells which have the capacity for self-renewal as well as differentiation. Recent studies support a cancer stem cell hypothesis in which these cells may be targets for mammary carcinogenesis. We have utilized both in vitro systems and mouse xenografts to investigate the mechanisms which regulate normal and malignant mammary stem cell self-renewal including components of the Hedgehog and Notch pathways. The Hedgehog signaling components PTCH-1, Gli1 and Gli2 are highly expressed in normal human mammary stem and progenitor cells cultured as mammospheres but are down-regulated when cells are induced to differentiate. Activation of Hedgehog or Notch signaling increases mammosphere initiating cell number and mammosphere size, whereas inhibition of this pathway results in a reduction of these affects. We demonstrate reciprocal interactions between Notch and Hedgehog signaling which are mediated by the polycomb gene Bmi-1. Stimulation of either Hedgehog or Notch pathways results in up-regulation of Bmi-1 with a resulting increase in mammosphere formation. In contrast, knockdown of Bmi-1 using a siRNA lenti-virus completely abrogates the stimulatory affects of Notch or Bmi-1 signaling. In mouse xenografts, over-expression of Gli2 in mammosphere initiating cells results in the production of ductal hyperplasia's, whereas inhibition of Bmi-1 inhibits mammary development. Both the Hedgehog and Notch pathways may be activated in breast tumor stem cells. These cells which we have previously characterized as CD44+ CD24 low lin- show increase activation of these pathways and Bmi-1 compared to the more differentiated tumor cells comprising the bulk of the tumor. These studies suggest that both Notch and Hedgehog signaling acting through Bmi-1 play an important role in normal and malignant stem cell self-renewal. Disregulation of normal stem cell self-renewal may result in expansion of stem cell pools which then are targets for further transformational events during carcinogenesis.