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SaP1
ADDITIVE ANTITUMOR EFFECT OF CONCURRENT TREATMENT OF 4-HYDROXY TAMOXIFEN WITH 5-FLUOROURACIL BUT NOT WITH DOXORUBICIN IN ESTROGEN RECEPTOR-α-POSITIVE BREAST CANCER CELLS
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Background: The sequential addition of tamoxifen (TAM) to chemotherapy seems superior to its concurrent addition in patients with breast cancer. This study was conducted to clarify the hypothesis that there are differential interactions among TAM and chemotherapeutic agents.
Methods: Estrogen receptor (ER) -α-positive or –negative breast cancer cells were treated with 4-hydroxy TAM (4OHT), 5-fluorouracil (FU) and/or doxorubicin (Dox). Changes in the expression levels of genes related to sensitivity and resistance to TAM, 5-FU or Dox were tested.
Results: Concurrent treatment of 4OHT with 5-FU but not with Dox additively inhibited the growth of ER-α-positive cells. 5-FU did not change the expression levels of any tested genes related to either sensitivity or resistance to TAM. Although Dox did not change the expression levels of any tested genes related to the sensitivity to TAM, Dox significantly increased the expression levels of genes related to TAM resistance, Eph-A2, ER-β, Fos and vascular endothelial growth factor. In addition, 4OHT significantly decreased thymidilate synthase (TS) activity.
Conclusion: The antitumor effect of concurrent 4OHT and 5-FU is additive, but that of concurrent 4OHT and Dox appeared to be antagonistic in ER-α-positive cells.
The increased expression of genes related to TAM resistance by Dox might be responsible for the antagonistic interaction. Decreased TS activity by 4OHT might increase the antitumor activity of 5-FU. These findings provide a preclinical rationale for concurrent use with 5-FU and TAM.

SaP2
COMBINED ANTITUMOR EFFECTS OF THE HER1/HER2 DUAL INHIBITOR, LAPATINIB WITH ANTIESTROGENS IN BREAST CANCER CELLS
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Background: Preclinical studies have suggested that activation of growth factor signaling induces estrogen-independent activation of estrogen receptor (ER) pathway and causes resistance to endocrine therapy in breast cancer. Inhibitors of growth factor signaling have been suggested to overcome such antiestrogen-resistance. To clarify this hypothesis, combined antitumor effects of the HER1/HER2 dual inhibitor, lapatinib with an antiestrogen (4-OH-tamoxifen or fulvestrant) were investigated in three human breast cancer cell lines, KPL-1, KPL-3C and KPL-4.
Methods: All the cell lines were established in our laboratory. The KPL-1 and KPL-3C cells are ER-positive and express low levels of HER1/HER2, and KPL-4 cells are ER-negative and HER2-overexpressed. Cell growth was measured using a Coulter counter. Cell cycle analysis was measured by flow cytometry. ER expression was measured by quantitative RT-PCR.
Results: The KPL-4 cells were most sensitive to lapatinib (the 50% growth inhibitory concentration: 2 μM). Lapatinib induced a massive apopotosis in the KPL-4 cells. Although antitumor effects of lapatinib were modest in the KPL-1 and KPL-3C cells, combined treatment with lapatinib and an antiestrogen resulted in an additive antitumor effect associated with an increase in apoptosis. In addition, a long-term exposure to lapatinib resulted in an

increased sensitivity to an antiestrogen in the KPL-1 and KPL-4 cells. Interestingly, up-regulation of ER expression by lapatinib was observed in the KPL-4 cells.
Conclusion: These findings suggest that lapatinib has a potent antitumor activity on HER2-overexpressing breast cancer and combined therapy (concurrent or sequential) with lapatinib and an antiestrogen may additively inhibit the growth of ER-positive breast cancer.

SaP3
PREDICTIVE FACTORS FOR RESPONSE TO COMBINED THERAPY WITH DOCETAXEL AND DOXYFLURIDINE IN PATIENTS WITH ADVANCED BREAST CANCER
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Background: Doxifluridine is activated to 5-fluorouracil (5-FU) by thymidine phosphorylase (TP). It has been suggested that docetaxel (DOC) up-regulates TP activity and combined treatment with DOC and doxifluridine synergistically inhibits the growth of human breast cancer xenografts. We conducted phase trials for combined treatment with intravenous bi-weekly DOC and oral doxifluridine in patients with advanced breast cancer. Predictive factors for response to this combination therapy were investigated.
Methods: A total of 19 patients with locally advanced or metastatic breast cancer were treated with intravenous bi-weekly DOC (40 mg/m2) and oral doxifluridine (600 mg/body daily). Response was evaluated according to the UICC criteria. Expression of BRCA1 and TP was analyzed by immunohistochemistry in primary breast cancer samples.
Results: The objective response (OR) rate to this combined therapy was 70.6% (12/17, response could not be evaluated in two patients). The OR rate was 90% (9/10) in BRCA1-positive tumors and 42.9% (3/7) in negative tumors (P = 0.112). The OR rate was 100% (8/8) in TP-positive tumors and 44.4% (4/9) in negative tumors (P = 0.045). In addition, the OR rate was 92.3% (12/13) in BRCA1-positive and/or TP-positive tumors and 0% (0/4) in BRCA1-negative and TP-negative tumors (P = 0.003).
Conclusion: This preliminary study has suggested that the combined treatment with intravenous bi-weekly DOC and oral doxifluridine is active in patients with advanced breast cancer and that the immunohistochemial analysis on BRCA1 and TP expression in breast cancer tissues may be predictive for response to this combined therapy.

SaP4
PRECLINICAL TESTING OF CHEMOPREVENTIVE AGENTS IN A MOUSE MODEL OF DCIS
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We have utilized a mouse model of DCIS for preclinical testing of chemopreventive agents. This model consists of transplantable premalignant mammary hyperplasia, or Mammary Intraepithelial Neoplasia-Outgrowth (MIN-O), originated from polyoma virus middle T (PyV-mT) transgenic mice. PyV-mT is a potent oncogene that is a molecular surrogate of ErbB RTKs and the PyV-mT mouse mammary tumor development mimic the biology of human breast cancer development. The MIN-Os have been maintained by serial transplantation into wild-type FVB host female mammary fat pads. The MIN-O mice, which carry transplanted MIN-Os in their no.4 mammary fat pads, develop invasive carcinoma at the transplant site at a predictable latency, and the growth of the lesion and the initiation of invasive carcinoma can be monitored with small animal mPET imaging.
We have utilized MIN-O mice for chemoprevention studies. So far, SERMs, an aromatase inhibitor, and an mTOR inhibitor have been tested for chemopreventive effects in trials ranging from 4 to 11-weeks. Aromatase inhibitor (arimidex) treatment had a slight inhibitory effect on MIN-O growth and tumor development at the early phase of treatment, but did not result in significant long-term inhibition of tumorigenesis. An mTOR inhibitor, rapamycin, effectively suppressed the MIN-O growth and significantly increased tumor latency from T50=46 to T50>86 days ($p<0.0001$). Rapamycin inhibited the activation of mTOR targets, and reduced cell proliferation and angiogenesis. However, the growth inhibitory effect was reversible, since the MIN-Os resumed growth after rapamycin withdrawal and eventually developed tumors. Gene expression analysis of the rapamycin treated MIN-Os identified the increased expression of PPARg related genes, suggesting a role for PPARg pathway in tumorigenesis in the MIN-O mice.

SaP5
NEW CHEMOTHERAPEUTIC DRUGS FOR TREATMENT OF BREAST CANCER BY MUTATION AND PROTOPLAST FUSION
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Chemotherapy is one of the main modalities in the therapy of breast cancer. Chemotherapy of breast cancer is limited by toxicity to normal cells and drug resistance of tumor cells. Chemotherapeutic agents mean any chemical compound or treatment method that induces cell damage and/or results in cell death. Anthracycline antibiotics are a mainstay of therapy for patients with metastatic breast cancer. Daunorubicin (DNR) and its C-14 hydroxylated derivative doxorubicin (DXR) or adriamycin are important antitumor anthracycline antibiotics isolated from Streptomyces peucetius ATCC 29050. Activity of the anthracyclines is related to topoisomerase II inhibition, which occurs as a result of anthracycline intercalation between adjacent DNA base pairs. Liposomal anthracyclines were developed to increase the therapeutic index of conventional anthracyclines by maintaining antitumor efficacy while improving the safety profile. Development of tumour cell resistance to anthracyclines involves a number of mechanisms, including P-glycoprotein-mediated resistance. New chemotherapeutic from anthracyclines drugs for cancer treatment have been traditionally originated by the isolation of natural products from different environmental niches, by chemical synthesis or by a combination of both approaches thus generating semisynthetic drugs. In the last years, a number of gene clusters from several anthracyclines biosynthetic pathways, mainly produced by actinomycetes and belonging to the polyketides family, are being characterized. Increased production of anthracyclines drugs, a very potent antibacterial antibiotic is achieved by medium and strain improvement. Improvement of antibiotic - producing microorganisms is achieved by several methods such as mutation, protoplast fusion and recombinant DNA technology. Novel anthracyclines derivatives have been produced by Mutation and protoplast fusion. Mutation and protoplast fusion are achieved randomly or rationally by change of regulation systems. It is possible to use of mutation and protoplast fusion in intensification of selection aimed at isolation of highly anthracyclines productive strains of genus streptomycetes.

SaP6
THE FIRST COMPREHENSIVE ANALYSIS OF THE BIOLOGICAL CHARACTERISTICS OF BREAST CARCINOMA TUMORS IN THE STATE OF KUWAIT
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Introduction: Breast cancer accounts for 30.3% of all cancer types in Kuwaiti females. A comprehensive analysis of the biological characteristics of the tumors was conducted in an attempt to determine any particular trend that could be present.
*Materials and Methods***:** Hundred and sixty six out of 200 patients whose paraffin blocks were available for evaluation were included in this study.
Results: Our results demonstrate that 70.5% of the patients were up to the age of 55. The mean age below 55 was 40 yrs as compared to 68 yrs above 55 ($P < 0.0001$).
The majority of the tumors were white or white and yellow, weighed between 50 and 500g, were either Solid/Fibrous-Schirrous or Solid in texture, and had irregular margins. Moreover, more than half of the cases were in the right breast and surgically treated by total mastectomy with axillary clearance.
The tumors were predominantly invasive of epithelial origin, Comedo/Necrosis for in situ and ductal for invasive carcinomas, had cancerization of the ducts, and a surrounding breast tissue of adenosis or fibrocystic type. In addition, the tumors had microcalcification of the ducts of vascular or vascular/perineuronal type, stromal elements, a mitotic index between 10 and 20 or >20, and a markedly or moderately enlarged nuclear polymorphism. They were grade II or III, sized 2-5 or > 5cm, had absent or scanty tumor lymphocytes, and were stage II or III.
Significant association was present between several above parameters and tumor expression of Her-2, ER, and PR.
Discussion: Breast cancer in the Kuwaiti population seems to be more aggressive than what is currently seen in Australia, North America, Europe, and some neighboring Arab countries.

SaP7

GENE EXPRESSION SIGNATURES OF HISTOLOGICALLY NORMAL BREAST TISSUE

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The role of the cellular microenvironment in breast tumorigenesis has become an important research area. However little is known about gene expression in histologically normal tissue adjacent to breast tumor tissue, and how this compares with non-tumor-bearing breast tissue. To address this, we have generated gene expression profiles of morphologically normal epithelial and stromal tissue, isolated using laser capture microdissection, from patients with breast cancer, or undergoing breast reduction mammoplasty. Based on these data, we determined that morphologically normal epithelium and stroma exhibited distinct expression profiles, but molecular signatures that distinguished breast reduction tissue from tumor-adjacent morphologically normal tissue were absent. Stroma isolated from morphologically normal ducts adjacent to tumor tissue contained two distinct expression profiles that correlated with stromal cellularity, and shared similarities with soft tissue tumors with favorable outcome. Adjacent normal epithelium and stroma from breast cancer patients showed no significant association between expression profiles and standard clinical characteristics, but did cluster ER negative, HER2 negative breast cancers with basal-like and normal-like subtype specific expression profiles. These subtypes over-expressed primarily epithelium specific genes. Our data reveal that morphologically normal tissue adjacent to breast carcinomas has not undergone significant gene expression changes when compared to breast reduction tissue, and provide an important gene expression dataset for comparative studies.

SaP8

ALTERATIONS IN GENE EXPRESSION BEFORE AND AFTER CARCINOGENIC STIMULI ON PAROUS MAMMARY GLANDS IN LEWIS RATS

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Early full-term pregnancy affords lifetime protection against the development of breast cancer. Parity-induced protection can be reproduced in a carcinogen-induced rat mammary carcinoma model. Oligonucleotide microarray analyses in parous and age-matched virgin (AMV) mammary glands of Lewis rats before and after N-methyl-N-nitrosourea (MNU) treatment were performed. Parous mammary glands before MNU treatment showed up-regulation of multiple differentiation-related genes, such as whey acidic protein (Wap), casein beta (Csn2), casein gamma (Csng), lipopolisaccaride binding protein (Lbp), secreted phosphoprotein 1 (Spp1) and glycosilation-dependent cell adhesion molecule 1 (Glycam1). Also, parous mammary glands before MNU treatment exhibited down-regulation of growth-related genes such as regenerating islet-derived 3 alpha (Reg3), mesothelin (Msln), insulin-like growth factor 2 (Igf2) and insulin-like growth factor binding protein 4 (Igfbp4). After MNU treatment, parous mammary glands exhibited down-regulation of growth-related genes, such as Msln, cell division cycle 2 homolog A (Cdc2a), Igf2, Igfbp4, stathmin 1 (Stmn1) and homeobox, msh-like 1 (Msx1), whereas expression of these genes remained high in AMV mammary glands. Parous mammary glands also exhibited marked down-regulation of Cdc2a and Stmn1 in response to MNU. The present data indicate that down-regulation of genes related to cell proliferation and cell cycle control (Reg3, Msln, Cdc2a, and Stmn1) may participate as key molecules in suppression of mammary cancer in parous rats.

SaP9
REDUCED P63 AND ELEVATED APOPTOSIS IN FOCALLY DISRUPTED MYOEPITHELIAL CELL LAYERS: EARLY SIGNS OF BREAST TUMOR INVASION?
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Our previous studies suggested that breast tumor invasion was triggered by a localized degeneration of aged or injured myoepithelial (ME) cells and the resultant auto-immunoreactions (Man et al. Breast Cancer Res 5:R231-241, 2003; Man and Sang. Exp Cell Res 301: 103-118, 2004; Man et al. Breast cancer Res Treat 89: 199-208, 2005). Our current study attempted to identify the early signs of ME cell degeneration. Consecutive sections were made from pre-invasive breast tumors with and without focally disrupted ME cell layers. The expression level of different ME cell-produced tumor suppressors and the frequency of apoptosis in focally disrupted and non-disrupted ME cell layers were statistically compared. Compared to their morphologically similar, non-disrupted counterparts, focally disrupted ME cell layers displayed several unique features, including: [1] a significantly lower expression of p63; [2] a significantly lower index of proliferation; [3] a significantly higher frequency of apoptosis; [4] a significantly higher frequency of leukocyte infiltration and degeneration. In sharp contrast, tumor cells overlying focally disrupted ME cell layers showed a significantly higher rate of proliferationand expression of tumor invasion-related genes, compared to their adjacent cells overlying non-disrupted ME cell layers. These findings suggested that reduction of p63 expression and elevation of apoptosis may represent early signs of ME cell degeneration and subsequent tumor invasion. Therefore, the development of therapeutic agents to prevent ME cell degeneration or to stimulate ME cell growth may provide a more effective approach for the treatment and prevention of breast tumor invasion.
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SaP10
GENOMIC ALTERATION AS A GUIDE TO THE DISCOVERY OF NOVEL THERAPEUTIC TARGETS IN BREAST CANCER
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Progression of breast cancer is associated with multiple genomic alterations including DNA copy number gains and losses. Some of these alterations contain important targets for therapy such as HER2. To further identify novel amplified ones, we applied high-resolution oligonucleotide-array comparative genomic hybridization (array CGH) on DNA from 50 breast tumors DNA. We simultaneously generated gene expression profiles using oligo expression arrays on the same sample sets. Statistical analysis of combined array CGH and cDNA data was performed to yield candidate oncogenes. The function of these putative novel oncogenes is being validated by gene knockdown experiments in breast cancer cell lines, and the clinical relevance is being assessed by clinically annotated breast tissue microarray (breast TMA). This direct comparison of the DNA and RNA-based tumor profiles will identify genes that may be novel targets for therapy in breast cancer.

SaP11
IDENTIFICATION OF COMMON PATHWAYS INVOLVED IN PUBERTAL MAMMARY GLAND DEVELOPMENT AND BREAST CANCER
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Developing mammary gland displays many properties which are also associated with breast cancer, such as invasion, reactivation of cell proliferation, resistance to apoptosis and angiogenesis. Conversely, many factors which have been implicated in breast cancer are essential for normal mammary gland development. Since more than 90% of human breast carcinomas derive from the ductal epithelium, it is essential to understand

the signals involved in normal development and function of the mammary ductal tree, and investigate their role in cancer. During puberty, the mammary gland develops by proliferation of the ductal epithelial cells, and invasion of these cells into the surrounding fat pad. Previous work by members of our group analysed the expression profile of mouse mammary glands during puberty, to identify the genes involved in this phase of rapid growth. This could prove a valuable starting point for identification of genes involved in pubertal mammary development, which may also be dysregulated in breast cancer. Several of the highly altered genes from this array were chosen for further analysis in human tissue, in particular in relation to possible roles in breast cancer. Among the genes selected were Claudin-4, a tight-junction protein involved in cell adhesion; CITED-1, a transcriptional coregulator involved in estrogen-dependent transcription; and Stanniocalcin-2, an estrogen-responsive secreted glycoprotein. We have carried out Western blot and immunohistochemical analysis for these proteins using breast cancer cell lines and breast tumour samples. Preliminary data for Claudin-4 suggests that it is expressed in 5 out of 6 of the breast cancer cell lines being studied. It is present at high levels in normal breast tissue. Antibodies for the other proteins are currently being optimised for staining. Experiments are in progress to examine a tissue microarray of 500 breast tumour samples for differential expression of these proteins, and determine any correlation with prognosis.

SaP12
EARLY RESPONSE OF MOUSE BREAST MINO MODEL OF HUMAN DCIS TO RAPAMYCIN

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Rapamycin, an immunosuppressant antibiotic, is a specific mTOR inhibitor capable of inducing G1 arrest and apoptosis in susceptible cancer cells. Six transplantable mouse precancerous (MINO) lines modeling human DCIS in the polyomavirus middle T transgenic FVB mouse have been biologically characterized for proliferation, tumor incidence, latency, and metastasis. The PI3K/Akt signaling pathway activated by the HER-2/ErbB2 family of growth factor receptors has been described in these lines, which become an ideal model for preclinical trial testing of the efficacy of cancer therapy drugs. It was observed that intraperitoneal application of 3mg/Kg, every other day for 7 days, was very effective in reducing the density, but not the percentage of fat pad occupied, of the MINO-B line. This was accompanied by numerous apoptotic figures. Six groups of 4 mice were cleared and transplanted with the MINO-B line and allowed to proliferate for 2 weeks (20-30% filled). Then the mice were treated IP with one dose of 3 mg/Kg rapamycin, and terminated at 1,3,6,12,24,and 72 hours post-treatment. Rapamycin had an effect on the density of the precancerous MINO-B line as early as 3 hours post-administration, but was most prominent after 72 hours. Histology demonstrated fewer epithelial cells with an increased number of apoptotic figures compared to controls. An increase of adipose cells was noted. Immunohistochemistry analysis demonstrated an increase of caspase 3 positive cells coincidental with the apoptotic figures; Ki67 showed no differences. Rapid apoptosis may be a result of molecular pathway disruption in a selected population of cells. Samples of the outgrowths were LN2 frozen for determination of gene expression by micro-array analysis.

SaP13
MOLECULAR CHANGES ASSOCIATED WITH MAMMARY TUMOR PROGRESSION IN A PYVMT-BASED MOUSE MODEL OF DCIS

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We have studied the changes found in the progression from normal to premalignant and ultimately to malignant stages in a novel mouse model of breast cancer. The model is based on mammary gland specific expression of PyVmT which induces rapid tumorigenesis.
Using oligo-array CGH analysis, we found that the most common chromosome aberrations in the premalignant tissues was a gain of chromosome 2, followed by chromosomes 11, 1, and 10. Some of the developing invasive carcinomas acquired additional genomic changes. Interestingly, 36% (5/14) of the premalignant tissues had no major genomic abnormalities. Gene expression analysis revealed dramatic expression differences between

normal mammary glands and premalignant tissues, including the corresponding expression increases of genes on the dysregulated chromosomes. Major changes were seen in the expression of cell cycle and apoptosis genes and components that are related to JAK/STAT, PI3K/Akt, and IL-2 pathways. Expression changes between the premalignant tissues and the corresponding invasive carcinoma tissues were less dramatic than the changes seen between the normal and the premalignant tissues. Moreover, there were no common genomic or gene expression changes associated with the 14 matching pairs of premalignant and invasive carcinoma.
These data suggest that mammary tumor progression is associated with common genetic changes at the premalignant stage, which include large chromosome aberrations and major gene expression changes in a common set of genes, whereas in the transition from premalignant to invasive carcinoma there are few additional and less dramatic genetic changes. Although all the lesions were derived from the same initial oncogenic event and acquired common basic molecular changes, premalignant lesions progress to have varying biological characteristics. This biological heterogeneity is very similar to that found in human premalignant lesions.

SaP14
HIGH RESOLUTION ARRAY CGH OF BREAST CANCER
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We have designed a Comparative Genomic Hybridization (CGH) array consisting of 244,000 in situ synthesized 60-mer oligonucleotides spanning the entire human genome. This results in an average genomic distance between probes of ~12 Kbp. Using these arrays, we performed an analysis of copy number changes in ERBB2 positive breast carcinomas and in breast carcinoma cell lines. Using the CGH analytics program we define multiple common aberrations within the sample set. We utilized a second, high definition array with >20,000 probes dedicated to Chromosome 17, with particular emphasis on the region encompassing ERBB2 (17q21.2-q21.3). Using these two arrays in combination, we provide a detailed description of genes that are co-aberrant with ERBB2 and discuss their potential contribution to the disease phenotype.

SaP15
GENE EXPRESSION PROFILE ANALYSIS OF MET RECEPTOR TYROSINE KINASE-INDUCED MOUSE MAMMARY TUMORS AND HUMAN BREAST CANCER
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Receptor tyrosine kinases (RTKs) have been shown to have an important role in the development and progression of breast cancer. The Met RTK, is involved in both normal mammary gland development and tumorigenesis. Overexpression of this receptor, as seen in 15-20% of human breast cancers, is an independent prognostic marker of poor prognosis and shorter disease-free survival. However, the role of a deregulated Met receptor in tumorigenesis is poorly understood. To examine the role of Met in mammary tumorigenesis, we have generated a mouse model that overexpresses an activated variant of Met under the control of the MMTV promoter. The activated Met variant induces mammary tumors with long latency (~367 days), heterogeneous histological patterns, and lung metastases. To understand the changes induced by Met in mammary neoplasia, we have undertaken a microarray approach. Since this murine model and human breast cancers produce tumors with cellular heterogeneity, Laser Capture Microdissection is used to enrich for isolation of epithelium, thus minimizing contamination from stromal and fat cells. Dissected epithelium from both tumor and matched normal is subjected to RNA isolation, amplification, labeling with fluorescent dyes, and microarray hybridization using Agilent Oligo arrays. Analysis has identified Met-induced gene expression changes during tumorigenesis by comparing Met tumor to matched-normal epithelial profiles. To establish whether a unique Met-specific profile exists, we have compared the Met-induced profile to those of other mouse models of mammary tumorigenesis (ie: Neu/ErbB2). Unsupervised hierarchical clustering has revealed a cluster of genes uniquely elevated and others downregulated in Met vs. ErbB2 tumors. To evaluate whether this model is representative of human breast cancers that overexpress Met, profiles from Met-induced tumors

will be compared to those of Met-overexpressing breast cancers.

SaP16
BREAST EPITHELIAL PROLIFERATIONS WITH BASAL AND LUMINAL PHENOTYPE ORIGINATE FROM P63-POSITIVE BASAL CELLS AND DEMONSTRATE DIFFERENT PATTERNS OF NOTCH RECEPTOR ACTIVATION

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We hypothesize that different types of progenitor cells are involved in the development of human intraductal/intralobular breast proliferations with basal and luminal phenotypes and this is accomplished via activation of different signaling mechanisms. Here we study the progenitor cells that participate in the development of usual ductal hyperplasia (UDH) and columnar cell change/hyperplasia (CCC/CCH) and evaluate Notch receptor activation using immunostains. We demonstrate that the luminal compartment of the human mammary gland consists of strongly CK18+ER+ (ER+CK18+CK14-CK5/6-EMA+SMA-p63-) and basal CK+ER- (ER-CK18-/+CK14+CK5/6+EMA+SMA-p63-) subpopulations that are phenotypically similar to CCC/CCH and UDH respectively. Rare p63+CK18+ cells were found in the strongly CK18+ luminal population of the normal gland, CCC/CCH, in the columnar component of the mixed lesions (CCH/UDH). p63+CK14+CK5/6+SMA-EMA- cells were found in UDH, and in the basal component of CCH/UDH. The examination of UDH at different stages of development showed that the proliferating basal cells originate from the p63 positive basal layer. Myoepithelial cells were Notch-2+ (nucleus/cytoplasm) Notch-3+ (nucleus/ cytoplasm)Notch-4+(membrane/cytoplasm). The CK18+ normal luminal cells, CCC/CCH and columnar component of CCH/UDH were Notch-2+(nucleus) Notch-3+(nucleus) Notch-4-. The estrogen-nonsensitive population of normal gland, UDH and basal component of CCH/UDH were Notch-2+(nucleus/cytoplasm)Notch-3+ (nucleus/ cytoplasm)Notch-4+(nucleus/cytoplasm). Our data suggests that the p63+CK18+ population is a progenitor of estrogen-sensitive luminal cells and CCC/CCH, while the p63+CK14+CK5/6+CK18-/+SMA- cell population participates in the development of UDH and may be a progenitor of the estrogen-nonsensitive luminal cells. In addition, we show that the activation of Notch-4 is involved in the proliferation of the basal cell population in UDH, whereas Notch-3 and Notch-2 participate in the development of both CCC/CCH and UDH. Thus, we outline two distinct differentiation pathways in the normal human mammary gland and benign proliferative breast lesions, which are probably implicated in the development of breast carcinomas with "luminal", "basal" or mixed phenotypes.

SaP17
A STROMA-RELATED GENE SIGNATURE PREDICTS SENSITIVITY TO EPIRUBICIN-CONTAINING NEOADJUVANT CHEMOTHERAPY IN BREAST CANCER: A MICROARRAY STUDY OF 102 PATIENTS INCLUDED IN EORTC 10994/BIG 00-01 TRIAL

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Background: In breast cancer patients the survival benefit of adding taxanes to anthracyclines in the adjuvant setting remains modest and is associated with additional toxicity and cost. Biological markers predicting sensitivity to anthracyclines would allow tailored chemotherapy treatment. Pathological complete response (pCR) is

a surrogate for chemosensitivity and the goal of this study was to identify a gene expression signature predicting for a pCR following neo-adjuvant anthracycline based chemotherapy.
Methods: The tumours used were from patients in the FEC arm (fluorouracil + epirubicin + cyclophosphamide x 6) of an ongoing randomized trial (EORTC 10994/BIG 00-01) which compares FEC with a taxane regimen in patients with large operable or locally advanced/inflammatory breast cancer. 102 samples (39 pCR) were hybridized to Affymetrix X3P arrays.
Findings: Among published predictive and prognostic signatures tested, only the Gianni signature was significantly better than chance at predicting pCR (AUC 0.64, p=0.01). Exploratory analysis using metagenes for biologically important processes identified stroma, T cells and interferon as potential predictive factors. In the ER negative tumour classes (basal and molecular apocrine), the stromal metagene had an AUC of 0.67 (95% CI 0.63-0.70), sensitivity 42%, specificity 86%, PPV 81% and NPV 51%.
Interpretation: Our results confirm the importance of immune-related genes and identify stroma as a factor influencing the sensitivity to FEC chemotherapy. These results support the activation of trials combining anti-stromal agents with chemotherapy.

SaP18
THE TRANSCRIPTION FACTOR GABP MAY PROVIDE A LINK BETWEEN OVEREXPRESSION OF HER2/NEU (ERBB2) AND DOWNREGULATION OF BRCA1
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The human breast cancer cell lines MCF-7, T-47D and SK-BR-3 express normal, moderately increased, and high protein levels of the proto-oncogene ErbB2 (Her2/Neu) respectively, and this is inversely correlated with expression of the GABP alpha/beta transcription factor. Data accumulated in our lab imply that loss of GABP is the result of decreased GABP beta subunit expression, the effect being most evident in the high ErbB2-overexpressing line SK-BR-3. As GABP appears to be a critical regulator of the BRCA1 gene, this correlation may be indicative of a functional link between ErbB2 overexpression and the loss of BRCA1. The observations that increased ErbB2 expression is correlated with extremely low BRCA1 levels and that ErbB2 amplification is not observed in familial BRCA1-linked breast cancers support this connection, the latter implying that BRCA1 loss is a downstream event of ErbB2 amplification. In order to determine if loss of the GABP complex is a common event in ErbB2-overexpressing breast cancers, we have performed immunohistochemistry staining of breast tumour tissue microarrays with antibodies against the GABP alpha and beta subunits. We are in the process of assessing the degree of staining of each section. Based on our results, we propose two models to link the ErbB2 pathway to BRCA1 expression. The first establishes a linear link between ErbB2 and BRCA1 that is GABP-mediated, while the second model places GABP in the position of key regulator of both the ErbB2 and BRCA1 genes.

SaP19
IDENTIFICATION AND ANALYSIS OF GENES PREFERENTIALLY EXPRESSED IN EPITHELIUM OR STROMA IN BREAST CANCER VS. NORMAL TISSUES
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The role of the cellular microenvironment in breast tumorigenesis has become an important research area. However little is known about gene expression in the tumor microenvironment. To address this, we have generated whole genome expression profiles of matched tumor epithelial and tumor stromal tissue as well as morphologically normal epithelium and stroma from patients with breast cancer (n=90). Specific tissue types were isolated using laser capture microdissection, and RNA was subjected to linear amplification. The results of analysis by class discovery and class prediction approaches to identify distinguishing features between tumor and normal cell types will be presented.

SaP20

IDENTIFICATION OF ANGIOGENESIS-ASSOCIATED GENETIC PROFILES USING LASER CAPTURE MICRODISSECTION AND MICROARRAY ANALYSIS

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Development of both primary solid tumors and distant metastasis depends on the formation of tumor vasculature. Tumor neovascularization (angiogenesis) usually results in tumor growth and the level of angiogenesis has been found to correlate with an increasing risk of metastasis and mortality in human breast cancer. A tumor "angiogenic switch" depends on the balance of pro- and anti-angiogenic molecules that can emanate from cancer cells, endothelial cells, stroma, blood and extracellular matrix. Presently, the identification of breast cancer-specific pro- and anti-angiogenic proteins is a challenge. To identify these proteins we have developed an approach (immunohistochemistry followed by Laser Capture Microdissection and microarray analysis) that allows us to isolate the epithelial, endothelial and connective tissue components from breast cancers and normal breast tissues taken from the same patient. Genetic profiles of each tissue can then be analyzed and the results correlated with the degree of angiogenesis and patient follow-up. Each dissected tissue is subjected to RNA extraction, linear amplification and microarray analysis. Real-time PCR confirms that microdissection is specific and results in nearly pure cell populations. Moreover, microarray data demonstrate the overexpression of tissue-specific markers as well as cancer-specific genes. Microarray analysis of gene expression in epithelium and stroma compartments for 25 patients belonging to the groups with high or low degrees of angiogenesis will be presented.

SaP21

APPLICATION OF IMMUNOHISTOCHEMICAL FEATURES WITH MULTIPLEX PCR FOR MUTATION DETECTION IN BREAST CANCER

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Aim: Breast carcinoma is the most common type of cancer occurring in women in Iran. BRCA mutations are associated with a greatly increased risk for cancer development. Archival materials are a valuable source for the study of molecular diagnosis methods in breast cancer and they are the most widely available material for retrospective clinical studies. We searched for BRCA mutations in women affected with breast cancer without having any prior knowledge of the family history, using immunohistochemical features as criteria.
Method: Immunohistochemical staining of sections from paraffin wax embedded tissues from these cases for the expression of ER, PR, HER-2 and p53 was carried out using the avidin biotin complex (ABC) procedure. Twenty-five different paraffin blocks with ER (-), and thirteen different tumor samples fixed on slides with ER (-) were used for DNA extraction and Multiplex PCR for detection of three mutation of BRCA1 and BRCA2 genes.
Results: Among the 100 cases with invasive ductal breast cancer selected in our study (20–85 age), 63%, 48%, 74%, and 61% were positive for ER, PR, p53 and HER2 respectively and no differences were observed between age groups for ER, PR, p53 and HER2 expression. The success of DNA amplification using simple boiling method was 76 % for paraffin blocks with ER (-), and 23 % for the tumor samples fixed on slides with ER (-). DNA extracted by the simple boiling method for paraffin blocks with ER (-), yielded higher proportions of successful gene amplifications (76%) than tumor samples fixed on slides (Difference, 53,00[18,81; 87,19], P=0,002). One of three mutations (5382insC) was found in 3 of the 38 archival samples of breast cancer patients.

SaP22
INVOLVEMENT OF HOXA5 GENE FUNCTION IN MAMMARY GLAND DEVELOPMENT AND NEOPLASIA

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Hox genes, which encode transcription factors, are involved in body patterning by instructing cells of their morphological fates according to their positions along the embryo axis. Misexpression of *Hox* genes is also associated with mammary gland neoplasia. For instance, misregulation of *HOXA5* expression was reported in breast carcinomas. How *Hox* genes participate to cancer is still unclear, but in this multistep progression where many molecular changes take place, inappropriate *Hox* gene expression may contribute to tumourigenesis. We have produced a Hoxa5 mutant mouse line. *Hoxa5*$^{-/-}$ mice present skeletal homeotic transformations and defects in organogenesis that impair viability of the mutant pups. Moreover, surviving *Hoxa5*$^{-/-}$ dams do not feed their pups properly due to lactation problems. Characterization of the *Hoxa5*$^{-/-}$ mammary gland phenotype revealed precocious lobulo-alveolar development as well as changes in proliferation and differentiation of the epithelium of nulliparous and pregnant *Hoxa5*$^{-/-}$ females preceding the abnormal secretory activity at parturition. Thus, the *Hoxa5* mutation perturbs mammary gland development and results in hyperplastic and mispecified mammary epithelium, suggesting that the loss of *Hoxa5* function may lead to a precancerous state. Using mammary epithelium grafting experiments, we also established that *Hoxa5* action on epithelium requires mesenchymal-epithelial crosstalk. Since deregulation of proliferation and differentiation are important events in the process of tumourigenesis, unravelling the signaling pathways responsible for *Hoxa5* action in the mammary gland should lead to a better understanding of the mechanisms underlying mammary gland development and tumour formation. To identify *Hoxa5* molecular targets in the mammary gland, different approaches have been used, including gene profiling. The results obtained will be presented and discussed.

SaP23
HEAT SHOCK PROTEIN-90ALPHA (HSP90ALPHA) IS ENCODED BY A PROLACTIN REGULATED GENE AND CHAPERONES ATAXIA-TELANGIECTASIA MUTATED PROTEIN (ATM) IN BREAST CANCER CELLS

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Elevated serum prolactin levels have been associated with an increased risk of human breast cancer (postmenopausal), and are causative in mice. Breast tumours also produce their own prolactin, increasing cellular exposure. Despite its important role, few target genes of prolactin have been identified in breast cancer. We identified the gene encoding the protein chaperone Hsp90alpha as a prolactin-Jak2-Stat5 target in breast cancer cells. Binding partners, or clients, of Hsp90 include many oncoproteins and prolactin-stimulated production of Hsp90alpha may therefore support oncoprotein function in breast cancer cells. The Hsp90 inhibitor 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) is currently being tested in clinical trials for breast cancer treatment. 17-AAG promotes degradation of Hsp90 client proteins that are improperly folded when Hsp90 is inhibited. To study Hsp90alpha function, we constitutively expressed the Hsp90alpha gene in the HC11 mammary epithelial cell line (Hsp90alpha-HC11). Hsp90alpha-HC11 cells respond to serum and hormone starvation with earlier and greater phosphorylation of histone 2AX than control cells. Histone 2AX is an ATM substrate and when phosphorylated is an indicator of DNA damage. We determined, using 17-AAG, that ATM is a client protein of Hsp90alpha in both HC11 and breast cancer cells, requiring Hsp90alpha for stability and function. We established a link between the prolactin pathway and Hsp90alpha to ATM, a key regulator of the DNA damage response pathway. Our results implicate Hsp90alpha in ATM activation, in ATM-based genomic stability of precancerous lesions and may also explain why 17-AAG treatment leads to radiation sensitivity in cancer cells.

SaP24
BREAST CANCER: KINASES AND PHOSPHOPROTEOME
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Due to the prominence of oncogenic kinases in various human malignancies, it is reasonable to speculate that cancer-specific phosphoproteomes exist. Here, we show that indeed, the tyrosine phosphorylation profiles of breast and liver tumors were distinct from each other. We have also identified a few proteins that were tyrosine phosphorylated in breast tumor. We have also conducted systematic characterization of breast cancer progression using cell line model. We demonstrated that breast cancer progression is accompanied by molecular changes in kinases such as Src and PI3K and that they have implications in cancer therapeutics. Our study highlights the myriad of signaling aberrations that challenge cancer therapy and that for individualized medicine to be realized (effectively), fingerprinting of key cancer-specific pathways is crucial.

SaP25
THE ROLE OF THE PROTEIN TYROSINE PHOSPHATASE PRL-2 IN BREAST CANCER
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The majority of breast cancer deaths result from tumor metastases rather than from primary tumors. Progress in understanding the biology of breast cancer metastasis has been limited by the lack of suitable experiment models. Because most oncogenes originally described were tyrosine kinases, protein tyrosine phosphatases (PTPs) were first postulated to be tumor suppressor genes. Yet, it is now clear that some PTP family members can also promote tumorigenesis. The PRL phosphatases (PRL-1, -2 and -3) constitute a novel class of small prenylated PTPs that have been proposed to possess oncogenic properties and to play a role in tumor metastasis. Interestingly, our data using human breast cancer cell lines show that PRL-2 is overexpress when compared to a normal breast cell line. We then studied the molecular and cellular function of PRL-2 by modulating its expression in these human breast cancer cell lines. In MDA-MB-231 cells, a highly metastatic breast cancer cell line, overexpression of PRL-2 amplified serum-induced migration, whereas silencing the PRL-2 gene using RNA interference decreased it. Overexpressing PRL-2 in MCF-7 breast cancer cells that are poorly metastatic allowed migration in response to serum. We are confident that this data will provide new insights into PRL-2 in breast cancer and will help to identify targets for inhibition of cellular transformation leading to metastatic breast cancer phenotypes.

SaP26
ROLE OF FOCAL ADHESION KINASE IN MAMMARY TUMORIGENESIS
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Focal Adhesion Kinase (FAK) regulates integrin-mediated adhesion and cell migration, and is involved in the control of cell proliferation and survival. Several reports have shown that FAK is over-expressed in different types of cancer, including breast cancer. However, there is no direct evidence whether, and if so how, FAK contributes to mammary tumorigenesis. For this purpose, we have introduced a conditional floxed fak allele into transgenic mice expressing the polyomavirus middle T (PyVMT) oncogene and the Cre recombinase (Cre), both under the transcriptional control of the mouse mammary tumor virus promoter/enhancer (MMTV). We initially showed that targeted ablation of FAK in the mammary epithelium does not impair mammary gland development. However, in PyVMT mice, whole-mount analysis revealed a dramatic reduction of hyperplastic mammary lesions in animals harboring two copies of the floxed fak allele (FAKflox/flox) as compared to controls. Interestingly, in FAKflox/flox mice, histochemical analysis showed that Cre-expressing cells are restricted to preneoplastic mammary epithelial regions but are not present in tumoral structures. We then used an adenovirus vector expressing Cre and GFP to infect primary cells cultured from mammary tumors and to monitor exclusively the Cre positive cells. Immunostaining experiments using antibodies against the proliferative marker Ki67 revealed an

impaired proliferative capacity of the FAK-null cells. Moreover, we observed a strong decrease in phosphorylated p60-Src kinase staining at the cell focal contacts. Taken together, these observations suggest that FAK expression is required for the progression from a preneoplastic to a tumorigenic phenotype in mammary tumorigenesis and that its role could be to favour the proliferation of PyVMT tumor cells through a p60-Src-dependent signaling pathway.

SaP27
ROLE OF CKIE MUTATIONS IN WNT SIGNALING AND BREAST CANCER
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Over 90% of breast cancers are non-familial and likely to result from somatic mutations in proto-oncogenes and tumor suppressor genes, many of which are unknown. We have identified candidate genes for a role in breast cancer based on their homology to tumor suppressor genes discovered in Drosophila. Most notably, 13% of breast cancer patients carried missense mutations affecting casein kinase I epsilon (CKIe), a Ser/Thr kinase that positively regulates Wnt signaling. Changes in the Wnt signaling pathway have been implicated in various cancers, particularly breast and colon cancer. However, the classical Wnt-activating mutations found in colon cancer are very rare in breast cancer, suggesting that other components of the pathway may be mutated. We are therefore testing the hypothesis that CKIe mutations activate Wnt signaling in breast cancer. We have used a Wnt-specific reporter assay (Topflash) to test the effect of the CKIe mutations on Wnt pathway activation, and preliminary results show slight but consistent differences compared to wild-type. In order to sensitize the assay, we are repeating it in the presence of Wnt3a, a CKIe activator. In addition, the role of CKIe mutations in mammary tumorigenesis is being investigated using both cell culture and in vivo models. We are testing transformation efficiency of each mutant combination by soft agar assay in MCF10A cells. We have also generated a mouse model in which the most promising mutant CKIe combination are expressed under the control of the mouse mammary tissue viral (MMTV) promoter using the Tet-On inducible system. This will allow us to study whether CKIe mutations are sufficient to induce tumors in vivo and/or whether they contribute to tumor progression.

SaP28
ROLE OF PTEN IN ERBB-2 MAMMARY TUMORIGENESIS
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Mice expressing an activated ErbB-2 under the control of its endogenous promoter in the mammary epithelium (ErbB-2KI) develop focal mammary tumors that show elevated levels of ErbB-2 generally due to genetic amplification of the activated ErbB-2 allele. Loss of PTEN has also been implicated in mammary tumor progression through mammary specific ablation of PTEN using a conditional allele. To directly assess the role of PTEN in ErbB-2 induced mammary tumorigenesis, we generated cohorts of transgenic mice females expressing an activated ErbB-2 under the control of its endogenous promoter and concomitantly homozygous or heterozygous for the loss of PTEN in the mammary epithelium (ErbB-2KI, PTEN-/- or ErbB-2KI, PTEN+/-). Mice heterozygous or homozygous for the PTEN deletion in the context of ErbB-2KI allele develop multifocal mammary tumors as early as 5 months and 8 weeks of age, respectively. Given that the ErbB-2KI and PTEN-deficient strains develop mammary tumors at 16 months and 10 months respectively, this suggests that loss of PTEN function can dramatically accelerate ErbB-2 induced mammary tumorigenesis. Elevated levels of ErbB-2 transcript and protein have been observed in these strains in the absence of datable ErbB-2 amplification. Immunohistochemical studies and western-blot analyses confirmed overexpression of ErbB-2 in these tumors. As expected, PTEN expression was lost in mammary tumors derived from the ErbB-2KI, PTEN-/-mice. Three out of seven mammary tumors collected from ErbB-2KI, PTEN+/- mice also showed loss of PTEN expression suggesting that loss of heterozygosity had occurred at the PTEN locus. However the other four still expressed the remaining PTEN allele suggesting that PTEN dosage may also effect tumor progression in this ErbB-2 model. In addition, ErbB-3, the preferred heterodimerization partner of ErbB-2, is co-overexpressed in those tumors. These data suggest that the generated mouse models will

provide an extremely useful tool to study the mechanisms underlying mammary tumorigenesis and metastasis.

SaP29
CHARACTERIZATION OF A HUMAN HER2 RECEPTOR SPLICE VARIANT
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Her2/ErbB2, a member of the ErbB family of receptor tyrosine kinases, is overepxressed in approximately 30% of breast cancer cases. In addition, activated forms of Her2 have been discovered. Targeted expression of an activated rat Her2 isoform in the murine mammary epithelium results in tumorigenesis with a latency of approximately seven months. Our laboratory has also discovered a naturally occurring human Her2 splice variant that, in lacking a 16 amino acid portion of the extracellular juxtamembrane domain, mimics the activated rat isoform. Since the domain lost in this splicing event is very close to the epitope targeted by the drug trastuzumab, we are interested in the signaling capacity of this splice variant. To study the signaling properties of this splice form, normal murine mammary gland (NMuMG) cells were stably transfected with both the wild type and splice forms of Her2. Analysis of the phosphorylation status of key signaling molecules downstream of Her2, such as AKT and MAPK, was assessed by western blot. It has also been observed that Her2 undergoes cleavage of the extracellular domain by an unknown matrix metalloproteinase. Using a combination of domain-specific antibodies, we determined that while still occurring in the wild type isoform, this cleavage event does not occur in the splice form of Her2. Hopefully, this study will provide insight into the mechanisms of Her2 cleavage and signaling as well as trastuzumab resistance.

SaP30
LOSS OF ONE OR BOTH TIMP3 ALLELES RESULTS IN MAMMARY TUMOR SUPPRESSION DUE TO PROMOTION OF EPITHELIAL APOPTOSIS
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Genetic suppressors of breast cancer are invaluable but elusive therapeutic targets. Here we show that tumorigenesis is inhibited in timp3+/- and timp3-/- mice in two independent models of breast cancer. Crossing timp3-/- with MMTV-PyMT mice resulted in greater than 90% reduction in tumor burden, multiplicity, and metastasis. Histological and molecular analyses showed that tumor initiation begins similarly across cohorts at puberty with the onset of tumor suppression at 60 days of age. Tumorigenesis in a non-viral oncogenesis model demonstrated similar breast cancer attenuation arising from Neu overexpression in both MMTV-Neu timp3+/- and MMTV-Neu timp3-/- mice. To determine which tissue compartment was responsible for tumor suppression, reciprocal mammary transplantation was performed. We found that stromal loss of timp3 was sufficient to trigger apoptosis within wild-type and timp3-/- tumors and curb malignant growth. To further address the molecular basis of this attenuation, we utilized cell lines from wild-type and TIMP3-null mammary epithelial tumors. Deficiency in TIMP3 sensitized tumor-derived cells to several apoptotic stimuli and serum withdrawal. Increased DNA fragmentation was accompanied by enhanced caspase 3 activity in timp3-/- primary cell lines. Thus, loss of one or both timp3 alleles potentiates apoptosis and results in significant mammary tumor suppression.

SaP31
LEF1 IS REQUIRED FOR TRANSITION OF WNT SIGNALING FROM MESENCHYMAL TO EPITHELIAL CELLS IN THE MOUSE EMBRYONIC MAMMARY GLAND
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Lef1-mediated canonical Wnt signaling is required for morphogenesis of skin appendages such as hair follicles and mammary glands during embryogenesis. In order to define the role of canonical Wnt signaling during early embryonic mammary gland development, we determined the temporal and spatial changes in Wnt signaling during embryogenesis in wild-type and Lef1-deficient embryos harboring a Tcf/Lef1-bgal reporter (TOPGAL) transgene. In contrast to previous studies using TOPGAL mice from a

distinct founder, we observe that Wnt signaling acts initially on mesenchymal cells associated with the sequential appearance of mammary placodes. As placode development progresses between 12.5 and 15.5 dpc, Wnt signaling progressively accumulates in the mammary epithelial compartment. By 18.5 dpc, bgal activity is confined to mesenchymal and epithelial cells near the nipple region. In Lef1-deficient embryos, the transition of Wnt signaling from mesenchyme to the mammary epithelia is blocked for placodes #1, 4 and 5 despite the expression of Tcf1 in epithelial cells. These placodes ultimately disappear by 15.5 dpc, while placodes 2 and 3 typically did not form in the absence of Lef1. Progressive loss of placodes 1, 4, and 5 is accompanied by increased apoptosis in mesenchymal cells adjacent to the mammary epithelial placodes. While factors important for embryonic mammary gland development, such as FGF7, are expressed normally in Lef1-deficient embryos, one mediator of the Hedgehog (Hh)-signaling pathway is aberrantly expressed. Both a receptor for and transcriptional target of Hh signaling, Ptc-1 expression is strongly reduced in mesenchymal cells surrounding the mammary placode in Lef1 mutants relative to wild-type embryos. The loss of Ptc-1 suggests that Hh signaling is blocked in Lef1-deficient embryos. Thus, these data reveal distinct requirements of different mammary placodes for Lef1-dependent Wnt signaling and further define dynamic changes in which cells integrate Lef1-dependent Wnt signaling during progression of embryonic mammary gland development.

SaP32

FHIT-PROTEASOME DEGRADATION IS CAUSED BY MITOGENIC STIMULATION OF EGFR-FAMILY MEMBERS

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The tumor suppressor gene FHIT is inactivated by genetic and epigenetic changes in the majority of common human cancers, including epithelial tumors such as breast and ovarian carcinomas. About one-third of human breast and ovarian carcinomas overexpress the oncogene HER2, inducing the activation of the EGFR-family pathways. Those HER2-overexpressing tumors result more aggressive. Src, a key cytoplasmic tyrosine kinase downstream to the EGFR-family, is able to phosphorylate the human Fhit protein on tyrosine residue 114. However, the biological significance of this phosphorylation has remained elusive. In the present study, we demonstrate for the first time that Fhit protein level is transiently reduced during cell proliferation mediated by activated tyrosine kinase receptors that recruit Src. Activation of EGFR-family members induce Fhit phosphorylation by Src and the subsequent proteasome degradation of the phosphorylated Fhit protein. During the signaling pathway of activated tyrosine kinase receptors, the reduction of Fhit protein level due to degradation of the phosphorylated Fhit allows the transmission of the mitogenic signal; immediately thereafter, Fhit protein levels are restored, suggesting a key role for Fhit in the balance of proliferation/survival/apoptosis signals. Therefore, the absence of FHIT in breast and ovarian tumors highly proliferating due to HER2 overexpression can further increase their aggressive phenotype by promoting their proliferation index.

SaP33

FUNCTION OF VEGF/VEGFR2 AND MAPK SIGNALING IN RESISTANCE TO ENDOCRINE BREAST CANCER THERAPY

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Endocrine resistance development is a major dilemma in clinical management of breast cancer (BC). Multiple molecular mechanisms are likely to contribute to such resistance. We and others have shown that high tumour tissue levels of vascular endothelial growth factor (VEGF) or its receptor VEGFR2 is a bad prognosis marker in BC patients treated with tamoxifen (Tam). Furthermore, we found that in patients with high VEGF/VEGFR2 expression, high expression of activated p38 negatively influenced patient survival. Here we examined how MAPK and VEGF signaling influence Tam responses. Using BC MCF-7 cell lines with or without Tam-resistance or with overexpression of VEGF, we have studied whether VEGF/VEGFR2 and MAPK signaling influence

responses to the ER antagonists 4-hydroxytamoxifen (4-OHT). We find that treating BC cells with the p38 inhibitor SB202190 increases the effect of 4-OHT treatment, suggesting that inhibition of p38 activity has a positive effect on killing these BC cells. In line with this we also find that cells resistant to 4-OHT display an increased level of p38. Similarly, the ERK inhibitor PD98059 increased the effect of 4-OHT treatment leading to increased cell death of both the tam sensitive and the tam resistant MCF7 cells. Moreover, our preliminary data indicates that VEGF-overexpressing MCF7 cells have increased basal activation of ERK1/2 compared to the parental MCF7 cell line. Taken together these results suggest that VEGF/VEGFR as well as p38 signaling likely regulate endocrine resistant in BC cells.

SaP34
REDIRECTION OF STAT3 ALTERNATIVE SPLICING AS A NOVEL ANTI-TUMORAL APPROACH

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Signal transducer and activator of transcription (STAT) proteins are a family of signaling molecules implicated in growth factors and cytokines signaling. STATs are latent transcription factors that are sequestered in the cytoplasm in an inactive form. Upon JAK/SRC dependent phosphorylation, they dimerize, translocate to the nucleus, and activate transcription of target genes. In normal physiological conditions, STAT proteins have a limited activation period whereas they (especially STAT1 and STAT3) show a persistent activation in many human cancers. This promotes growth and survival of tumor cells, induces tumor angiogenesis and suppresses anti-tumor immune responses. Because of its pivotal position at the convergence of many oncogenic tyrosin-kinase signaling pathways, STAT3 seems to be particularly suitable as a molecular target for cancer therapy, especially considering that tumor cells tend to become addicted to persistent STAT3 signaling. A naturally occurring alternative splicing variant, STAT3-beta, uses an alternative acceptor site within exon 23 and leads to the production of a truncated isoform, which lacks the C-terminal trans-activation domain (TAD). STAT3-beta can act as a dominant negative regulator of transcription and promote apoptosis. We are characterizing the cis-elements and trans-acting factors that regulate STAT3 exon 23 alternative splicing and have used modified antisense oligonucleotides to specifically induce a shift from the abundant, active STAT3-alpha to the anti-tumorigenic STAT3-beta isoform derived from transfected reporter minigenes or from the endogenous gene in multiple breast cancer cell lines.

SaP35
THE CRITICAL ROLE OF TYROSINE PHOSPHORYLATION OF GRB2 IN PROLACTIN INHIBITORY EFFECTS ON EGF-INDUCED MAPK PATHWAY IN MAMMARY EPITHELIAL CELLS

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Both prolactin (PRL) and epidermal growth factor (EGF) are important factors regulating mammary epithelial cell growth and differentiation. While EGF is a potent mitogen, PRL is critical for induction of functional differentiation of mammary epithelial cells. Here we evaluated the role of PRL signaling in the regulation of EGF-induced mitogen activated protein kinase (MAPK) Erk1/Erk2 pathway in mammary epithelial cells. Treatment of HC11 cells with PRL led to a mild activation of the MAPK Erk1/Erk2 pathway in comparison to EGF stimulation. Moreover, our results indicate a PRL inhibitory effect on EGF-induced MAPK activation. This was found to be upstream of Ras/Raf interaction suggesting a grb2/Sos dependent mechanism. Indeed tyrosine phosphorylation of grb2 on residues Y7, Y37, Y52 and Y209, previously implicated in modulating grb2/sos interactions, was observed following PRLR activation. Furthermore, treatment of mammary epithelial cells with PRL but not with EGF led to a significant increase in tyrosine phosphorylation of grb2. Using a mutant form of grb2 lacking the potential tyrosine (Y) phosphorylation sites (Y7, Y37, Y52 and Y209) we showed a significant recovery in EGF-induced MAPK (Erk1/Erk2) activation and Ras/Raf interaction in the presence of PRL. Our data suggest that PRL-induced tyrosine phosphorylation of grb2 will lead to impairment in grb2/Sos interaction resulting in attenuation of the Ras/MAPK cascade downstream of the EGFR. These results implicate PRL-induced tyrosine phosphorylation of grb2 as a critical mechanism by which differentiated

mammary epithelial cells may escape from the mitogenic effects of EGF.

SaP36
CROSS-REGULATION OF INHIBITOR OF APOPTOSIS PROTEINS (IAP) AND TAK1 AND THEIR ROLES IN BREAST CANCER CELL SURVIVAL
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Inhibitors of apoptosis proteins (IAPs) are a family of caspase inhibitors include cIAP1, cIAP2, the X-chromosome-linked IAP (XIAP) and survivin. Overexpression of XIAP and survivin correlate with poor prognosis and resistance to cancer chemotherapy in humans and small molecules and peptides targeting XIAP and survivin sensitize cancer cell death upon stimulation of TNF-related apoptosis-inducing ligand (TRAIL) and therapies targeting IAPs are now in the clinic. The role of IAPs function as caspase inhibitors has been intensely studied but this family of molecules has several other functions that have not been well characterized. For example, cIAP1, cIAP2 and XIAP have been implicated in JNK and NF-kB signaling events but their precise roles in this pathway have not been clearly elucidated. Furthermore, these IAPs all have the potential to act as E3 ubiquitin ligases and the physiological targets and roles of this function remains uncertain. The TAK1 mitogen-activated protein kinase kinase kinase has recently emerged as a key enzyme involved in activation of the NF-kB and Jun kinase signaling cascades. XIAP has previously been implicated in TAK1 function and we are testing the hypothesis that a cross-regulatory complex of cIAP1, cIAP2 and XIAP functions to regulate levels and activity of TAK1. Using RNA interference to knockdown cIAP1, we have demonstrated an increase in the levels of cIAP2, XIAP and TAK1 in MDA-MB-231 breast cancer cells. Conversely, knocking down XIAP levels with RNA interference results in reduced levels of TAK1. Thus, there is substantial cross-regulation between these IAPs and TAK1 in breast cancer cells. We intend to utilize the RNA interference technique and mouse embryonic fibroblasts from IAP knockout mice to further examine the cross-regulation between the IAPs, their interaction with TAK1, and the importance of these events in cancer cell survival.

SaP37
THE ROLE OF AKT2 IN MAMMARY GLAND DEVELOPMENT AND TUMOURIGENESIS
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The Akt serine/threonine kinase has been implicated as an important signaling molecule in the progression of cancer. It has become increasingly clear that the different Akt isoforms are involved in distinct processes and regulate unique target proteins. To further evaluate the role of the Akt family in mammary gland development and tumourigenesis, we have generated transgenic mice expressing a constitutively active Akt2 (T309D/S474D or Akt2-DD) in the mammary epithelium. No defects in mammary gland development in these transgenic mice have been noted, nor does the expression of activated Akt2 on its own lead to mammary tumourigenesis. Consistent with the important role of Akt as a negative regulator of apoptosis, the expression of an activated Akt2 results in a delay in mammary gland involution following weaning of the pups. To examine the role in mammary tumourigenesis, the Akt2DD mice have been interbred with transgenic mice expressing a mutant Polyoma Virus Middle T oncogene which is uncoupled from the PI3 K pathway (MT Y315/322F). The co-expression of Akt2-DD does not affect mammary tumour onset, however it does result in an increase in secondary metastases to the lungs of tumour bearing mice. In support of this observation, the infection of a non-metastatic mouse mammary tumour derived cell line with a retrovirally expressed Atk2-DD results in an elevation of invasion in an in vitro invasion assay and results in leads to an increase in metastases to the lungs when injected into the fat pad of nude mice. The results of this study will help to further elucidate the contribution of the different Akt family members in mammary gland development and mammary tumourigenesis.

SaP38
ROLE OF JAB1 IN RESISTANCE TO TRASTUZUMAB (HERCEPTIN) TREATMENT
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Recent findings indicate that breast carcinoma cells that overexpress HER2 proliferate at a higher rate because p27Kip1, a cell cycle inhibitor of Cdk2 and cell proliferation, remains in the cytoplasm and does not translocate to the nucleus. This loss of translocation of p27 occurs when AKT phosphorylates p27 on threonine 157 (T157), which is in the nuclear localization domain of p27, thus destroying the sequence necessary for translocation. Trastuzumab (Herceptin), a recombinant humanized anti-HER2 monoclonal antibody (Genentech, South San Francisco, CA) increases the nuclear p27 protein level by inhibiting AKT and the resulting phosphorylation of p27, which allows p27 to enter the nucleus. Once in the nucleus, p27 inhibits Cdk2 activity, and the cells remain in the G0/G1 phase of the cell cycle. Inhibition of PI3K and AKT by PTEN may also contribute to tumor inhibition by Herceptin. Herceptin-resistant cells retain HER2 amplification and overexpression as well as the ability to bind Herceptin. However, activation of AKT is downregulated by Herceptin independent of Herceptin sensitivity. Interestingly, expression of p27 restores Herceptin sensitivity to parental and resistant cells. Thus, reduced levels of p27 may be a major mechanism of Herceptin resistance. Although JAB1 is known to increase p27 ubiquitination and degradation and downregulate cyclinE-Cdk2 kinase activity, the effect of JAB1 on HER2 signaling has not been studied. We hypothesize that JAB1 overexpression in breast cancer plays a major role in resistance to Herceptin treatment and that inhibition of JAB1 may enhance sensitivity to Herceptin treatment by preventing nuclear-cytoplasmic shuttling of p27 by JAB1. If our hypothesis is correct, combination treatment of HER2-positive breast cancer cells with Herceptin and JAB1 inhibitors may represent a valid therapeutic option that will sensitize drug-resistant cells to Herceptin treatment.

SaP39
THE ROLE OF 14-3-3SIGMA IN THE ERBB2-MEDIATED MAMMARY TUMORIGENESIS
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14-3-3σ is a putative tumor suppressor expressed exclusively in epithelial cells and is transactivated by p53 in response to DNA damage. Its over-expression can enforce a G2/M cell cycle arrest, while its disruption leads to mitotic catastrophe. Epigenetic silencing of 14-3-3σ has been detected at a high frequency in breast carcinomas. The ErbB2 protoncogene belongs to the EGFR family and is essential to cell proliferation and differentiation. Amplification and overexpression of ErbB2 is observed in 20-30% human mammary carcinomas. Comparative genomic hybridization assays of ErbB2-mediated mouse mammary tumors revealed a recurring loss of a 1.88Mb fragment on chromosome 4, which contains the 14-3-3σ gene. In this study, we further discovered an inverse correlation between ErbB2 and 14-3-3σ protein levels in our ErbB2 knock-in tumors as well as in a number of independent human breast epithelial cell lines. Over-expression of 14-3-3σ in a mouse mammary tumor cell line TM15, which was derived from one of the ErbB2 knock-in tumors, lead to the down-regulation of ErbB2 and restoration of the disrupted cell junctions observed in the parental TM15 cells. These results suggest that 14-3-3σ may negatively regulate ErbB2 and play a positive role in promoting cell junction and polarity.

SaP40
XIAP IS A TARGET OF TAK1 ACTIVITY THAT SUPPRESSES TRAIL-INDUCED APOPTOSIS
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The TAK1 mitogen-activated protein kinase kinase kinase is required for stimulus-induced activation of the NF-kB and Jun kinase signaling cascades. NF-kB activation plays a crucial role in facilitating transcription of pro-survival genes and in this study, we have addressed whether TAK1 activity confers resistance to apoptosis in MDA-MB-231 breast cancer cells and in HEK293T kidney cells. Using RNA interference to reduce TAK1 levels, we show that TAK1 plays an important role in the maintenance of survival in both cell lines and demonstrate that TAK1 activity confers resistance to TRAIL-induced apoptosis. TAK1 is shown to play a crucial role in basal and induced NF-kB activation and XIAP is identified as a key downstream target of TAK1 that facilitates survival responses. Taken together, these data highlight a role for TAK1 in promoting the survival of transformed cells by facilitating the accumulation of XIAP.

SaP41
IDENTIFICATION OF MULTIPLE ISOFORMS OF KLF4/GKLF AND THEIR DIFFERENTIAL REGULATION BY ERBB2 AND TGF-β IN HUMAN BREAST EPITHELIAL CELLS

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Amplification of ErbB2 (HER2/Neu) occurs in 20-30% of human breast cancers. While ErbB2-targeted therapies such as Trastuzumab have been effective in treating this subset of breast cancers, many tumors are resistant, requiring identification of additional targets for therapeutic development. We have identified Gut-enriched Krüppel-like factor (KLF4/GKLF) as one of these potential targets. KLF4 is a zinc-finger transcription factor whose decreased expression in colorectal and prostate cancers supports a role for promoting differentiation and inhibiting proliferation of epithelial cancers. However, recent studies have shown that KLF4 expression is increased in aggressive human breast cancers. To determine if KLF4 is expressed in HER2/Neu positive human breast cancers, we performed immunohistochemical analyses. KLF4 is expressed in regions of active ErbB2 signaling in these tumors, suggestive of a tumor promoting role for KLF4. We also found that KLF4 protein expression increases with Heregulin activation of ErbB2 signaling in MCF-7 cells, while the ErbB2 selective inhibitor, AG825, decreased basal KLF4 expression in ErbB2-amplified BT-474 breast cancer cells. These data indicate that KLF4 may be an integral regulator of transcriptional changes induced by ErbB2. While performing these studies, we identified two isoforms of the KLF4 protein. Expression of the larger isoform is stimulated by ErbB2 activation, while the shorter isoform remains unchanged. In contrast, TGF-β treatment of MCF-10A cells inhibits expression of the shorter isoform without affecting expression of the larger isoform. Current studies are aimed at identifying the mechanisms of differential regulation by ErbB2 and TGF-β as well as determining the functional roles of each KLF4 isoform in regulating tumor promotion or suppression.

SaP42
BREAST TUMOR KINASE (BRK) TARGETS KINESIN-ASSOCIATED PROTEIN 3 AND ENHANCES CELL MIGRATION

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BRK (BReast tumor Kinase) is a non-receptor tyrosine kinase overexpressed in 60% of human breast tumors, including lymph node metastases, but undetected neither in normal mammary tissue nor in fibroadenomas. The incidence of BRK in breast cancers is significantly higher than that of the HER2 overexpression which is between 25-30% or p53 mutation which is about 20%. Therefore, BRK can be postulated as a more relevant marker and target for poor prognosis and breast cancer therapy respectively. Studies have suggested that BRK is involved in mitogenic signaling and cell proliferation; however, the intracellular roles of BRK have not been fully elucidated. RNA-binding protein Sam68, paxillin, insulin receptor substrate-4 and breast tumor kinase substrate (BKS) are the only known substrates of BRK. We show that overexpressed activated BRK phosphorylates several cellular substrates. Thus to understand the cellular roles of BRK and delineate the signaling pathways regulated, we sought to identify new BRK targets. We have performed large-scale kinase assays with active recombinant GST-BRK and [32P]-γ-ATP on high-density protein filter arrays and obtained 20 hits, five of which were validated as legitimate BRK substrates. They include proteins implicated in the cytoskeletal dynamics such as kinesin-associated protein 3 (KAP3), beta-tubulin, cofilin 1 and angio-associated migratory cell protein (AAMP) as well as G protein alpha-subunit GNAS. We confirm that KAP3 associates with and is phosphorylated by BRK on C-terminal tyrosine residues. Finally, we demonstrate that BRK-phosphorylated KAP3 enhances cell migration.

SaP43
CONSEQUENCES OF DDR1 COLLAGEN-RECEPTOR OVEREXPRESSION IN HUMAN BREAST CANCER

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The interaction between malignant cells and the extracellular matrix plays an important role in directing tumor growth, invasion and metastasis. Our previous work showed that discoidin domain receptors (DDRs) are a family of tyrosine kinase receptors that are activated by native, triple-helical collagen. Of importance, DDR1 is expressed in epithelial cells including the mammary gland epithelium and is overexpressed in ductal breast carcinomas. The lack of DDR1 in knockout mice results in severe defects during lactation. From the 5 isoforms currently known of DDR1, isoforms a, b and c are active enzymes while DDR1d and DDR1e are kinase-dead isoforms. Upon activation of its catalytic function, a number of cytoplasmic signaling molecules, such as ShcA, Nck2 and Shp-2, bind DDR1 in a phosphotyrosine-dependent manner. To investigate the role of DDR1 in human breast cancer, we stably expressed the a-, b- and d-isoform in the metastatic breast cancer cell line MDA-MB-231, which lacks endogenous DDR1 expression. Cell clones overexpressing full-length DDR1 responded with sustained receptor activation upon collagen stimulation. We determined adhesion to collagen matrixes and proliferation within a collagen-rich microenvironment in vitro. To examine its function in vivo, DDR1-overexpressing cells were intracardially injected or orthotopically implanted into to the mammary fat pad of immuno-compromised mice. Tumor cell growth and dissemination as well as the formation of bone metastases was monitored twice per week in anaesthetized animals using the Kodak Image2000 station. We observed a higher incident of bone tumors in mice injected with cells overexpressing DDR1b than control-transfected cells. Volumetric computed tomography (VCT) as well as Faxitron analysis were used as alternative methods to quantify tumor load and bone metastasis. Our data provide compelling new evidence that DDR1 regulates proliferation of human breast tumor cells within a 3D microenvironment as well as metastasis in an experimental mouse tumor model.

SaP44

ILK IS REQUIRED FOR EFFICIENT ERBB2/NEU-INDUCED MAMMARY GLAND TUMOURIGENESIS

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The MMTV-neu model of breast cancer was used to elucidate the role of ILK in mammary tumourigenesis. Previously, an oncogenic role for ILK was confirmed in vitro and in vivo. In addition, ILK has been shown to be activated downstream of the b1-integrin receptor, which was shown by our laboratory to be necessary for mammary tumourigenesis in vivo. ILK facilitates cross-talk between growth factor receptors and cell adhesion receptors, inducing the activation of survival and proliferation pathways. These properties of ILK reflect its role as an important component of cell adhesion complexes, which are recognized as critical mediators of tumour progression. Since these complexes include growth factor receptors such as neu, we wanted to determine if the loss of ILK could impact mammary gland tumourigenesis in the MMTV-neu model. For this purpose we used a Cre/Lox approach to ablate the ILK coding sequence from the mammary epithelium of MMTV-neu mice. Following ablation of ILK, we observed a dramatic delay in neu-induced tumour onset. Interestingly, tumours arising in ILK-/- mice appeared stochastically, in contrast to global transformation of the mammary epithelium in control MMTV-neu mice. TUNEL analysis of glands from MMTV-neu ILK-/- mice revealed extensive apoptosis, which was shown to be associated with a reduction in Akt phosphorylation. Immunoblot analysis of tumours arising in MMTV-neu ILK-/- mice revealed activation of the NFkB pathway, which was confirmed by nuclear staining for NFkB in histological sections. Together, these results suggest that ILK is necessary for efficient mammary tumourigenesis in MMTV-neu mice. In addition, these results suggest that activation of the NFkB pathway can compensate the loss of ILK/Akt-mediated cell survival during mammary tumourigenesis.

SaP45
THE JANUS KINASE 2 (JAK2) IS REQUIRED FOR EXPRESSION AND NUCLEAR ACCUMULATION OF CYCLIN D1 IN PROLIFERATING MAMMARY EPITHELIAL CELLS
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Using a conditional knockout approach, we previously demonstrated that the Janus kinase 2 (Jak2) is crucial for prolactin signaling and normal mammary gland development. Prolactin is suggested to synchronously activate multiple signaling cascades that emerge on the prolactin receptor. This study demonstrates that Jak2 is essential for the activation of Stat5 and expression of Cish, a Stat5-responsive negative regulator of Jak/Stat signaling. Jak2, however, is dispensable for the PRL-induced activation of c-Src, Fak, and the MAPK pathway. Despite activation of these kinases that are commonly associated with proliferative responses, the ablation of Jak2 reduces the multiplication of immortalized mammary epithelial cells. Our studies show that signaling through Jak2 controls not only the expression of the Cyclin D1 gene but, more importantly, it regulates the accumulation of Cyclin D1 in the nucleus by inhibiting the phosphorylation, subsequent nuclear export, and degradation of Cyclin D1. Consistent with this observation, the levels of activated Akt and phosphorylated GSK3beta are reduced in Jak2-deficient MECs. The proliferation of Jak2-deficient MECs can be increased by expressing of a mutant form of Cyclin D1 that cannot be phosphorylated by GSK3beta and therefore constitutively resides in the nucleus. Besides discriminating Jak2-dependent and Jak2-independent signaling events emerging from the prolactin receptor, our observations provide a possible mechanism for phenotypic similarities between Cyclin D1 knockouts and females lacking individual members of the PRLR signaling cascade, in particular the PRLR, Jak2, and Stat5.

SaP46
THE ROLE OF THE JANUS KINASE (JAK2) DURING ONSET AND PROGRESSION OF MAMMARY TUMORIGENESIS
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Jak2 is a hormone-receptor-coupled kinase that mediates the tyrosine phosphorylation and activation of signal transducers and activators of transcription (Stat). The biological relevance of Jak/Stat signaling during normal development of adult tissues and neoplastic transformation is difficult to investigate since Jak2 deficiency leads to early embryonic lethality. We therefore generated Cre/lox-based Jak2 conditional knockout mice and found that this kinase is indispensable for normal mammary gland development during pregnancy. We now demonstrate that Jak2-deficiency confers resistance toward Her2/neu-mediated neoplastic transformation of mammary epithelial cells, suggesting that targeting Jak2 before tumor onset is a relevant strategy for cancer prevention. One advantage of a Cre/lox-based genetic model to study the function of a gene during tumorigenesis is that we are able to modify Jak/Stat signaling both prior to growth factor-mediated neoplastic transformation and during particular stages of the progressing disease. Despite its role for cancer prevention, the retroviral Cre-mediated deletion of Jak2 from cancer cells did not affect the survival and proliferation of neoplastic Jak2-/- cells in culture, suggesting that targeting only Jak2 may not be a suitable strategy for cancer therapy of breast cancers expressing Her2/neu. Ongoing studies focus on the in vivo growth properties (i.e. tumor cell proliferation and metastasis) of Her2-overexpressing, Jak2-deficient mammary cancer cells and their isogenic Jak2-expressing controls. For this purpose, we orthotopically transplanted luciferase-tagged Jak2-deficient breast cancer cells and their controls into the mammary gland of recipient females and use a bioimaging tool (IVIS200, Xenogen) to monitor tumor growth and the formation of distant metastases in individual animals.

SaP47
CHARACTERIZATION OF ERBB2 TYROSINE AUTOPHOSPHORYLATION SITE MUTANTS
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ErbB-2 overexpression and amplification is observed in 20 - 30% of human mammary carcinomas and correlates with poor clinical outcome. We have previously demonstrated that

four ErbB-2 tyrosine autophosphorylation sites (YB, YC, YD and YE) within the cytoplasmatic tail are sufficient to mediate transforming signals in vitro. These autophosphorylation sites have been shown to bind distinct adapter proteins including Grb2, Shc, and Crk, suggesting that ErbB-2-mediated transformation functions through numerous distinct effector pathways. To address the biological importance of each individual tyrosine autophosphorylation site in ErbB-2-mediated mammary tumorigenesis, we derived a variety of transgenic mice expressing mutant ErbB-2 receptors in the mammary gland, which posses only a single functional autophosphorylation site while all others were mutated to phenylalanine residues. Recently, we showed that YB and YD female transgenic mice rapidly developed mammary tumors with differences in tumor latency, morphology, and metastatic potential. To further understand the role of the autophosphorylation sites in ErbB2-induced mammary tumorigenesis, we characterized the YC and YE transgenic mouse model. Our data showed that YC and YE female mice developed multifocal tumors after a short latency similar to the latency period obtained for YD transgenic mice. The morphology of YC and YE mammary tumors were similar to the morphology observed in YD mammary tumors. Interestingly, the percentage of YC and YE female transgenic mice with lung metastasis was similar to the metastatic potential observed in YB transgenic mice. Although, mammary tumors derived from the YC and YE transgenic mouse model have similar phenotypes, they do differ in latency, morphology and metastatic rate compared to the YB and YD transgenic mouse model suggesting that the recruitment of specific adaptor proteins has a distinct biological effect on ErbB2-mediated mammary tumorigenesis.

SaP48
17 BETA ESTRADIOL AND TAMOXIFEN, BUT NOT ICI 182 780 STIMULATED RAPID TRANSIENT ACTIVATION OF ERK IN MCF - 7 CELLS
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Estrogen receptor (ER) positive breast cancers, comprising 65% of breast cancer cases, are suitable for hormone treatment. While the different forms of hormonal treatment available vary in their mechanisms of action, they all strive to diminish estrogen signaling in ER positive cancer cells. Traditionally, estrogen signaling was thought to be restricted to nuclear mechanisms involving transcription from genes regulated by the ER. Recently, 17β estradiol, a form of estrogen, was shown to induce rapid activation of the MAP kinase family members ERK1 and ERK2. While the exact mechanism of action is not understood, these findings indicated that estrogen signaling might not be limited to nuclear events mediated by ligand engaged ER. These observations underscore the importance of understanding the effects of compounds which are thought to specifically target estrogen signaling on other pathways which might influence the survival or proliferation of breast cancer cells.
We have used the ER positive human breast carcinoma cell line MCF-7 to investigate ERK activation in response to 17β estradiol, or the hormonal agents tamoxifen citrate and ICI 182 780 (fulvestrant). Serum starved cells were challenged with these agents and ERK activation was assessed by immunoblotting. Both 17β estradiol and tamoxifen citrate caused a rapid and transient activation of ERK. In contrast, ICI 182 780 did not activate ERK. These results demonstrated that tamoxifen, which is designed to reduce estrogen signaling in ER positive cells, could also activate other pathways that are responsive to estrogen. Furthermore, we show that an alternative estrogen-antagonist, ICI 182 780, did not activate ERK. ICI 182 780 has been clinically shown to be effective in tamoxifen resistant breast cancer cases, and our results provide a mechanistic basis for this observation.

SaP49
THE BRCA1 GENE: A POSSIBLE LINK BETWEEN STRESS AND BREAST CANCER
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The molecular pathways linking psychological stress to breast cancer development are not well understood. We have identified one such possible pathway involving the tumour suppressor gene BRCA1. Experiments from our lab have shown that BRCA1 expression in breast cells is down-regulated in response to treatment with the stress hormone hydrocortisone in mice in vivo and in vitro. Since BRCA1 down-regulation occurs in most sporadic breast cancers, this could represent an important link

between stress and breast cancer development. The effect observed is concentration dependent and correlates with length of exposure, but is greatly reduced in mouse and human tumour cell lines. It is also dependent on the growth and differentiation state of the cell as shown in cells grown both on plastic and on a synthetic basement membrane-like substrate. The hydrocortisone effect is conferred via specific BRCA1 promoter sites including UP and BRIBS, which show loss of a high molecular weight protein complex in response to treatment and which appear to regulate BRCA1 expression in a cooperative manner. Two of the proteins forming part of the UP and BRIBS complexes were identified as the transcription factors USF2 and GABPα/β. A third regulatory site within the promoter seems to be necessary for full BRCA1 repression by hydrocortisone. A likely candidate is the BrCREO promoter element which shows binding of the transcription factors c-Jun and FRA2. c-Jun is part of the AP-1 complex known to be co-regulated and to interact with the glucocorticoid receptor.

SaP50
GABP AS A POTENTIAL LINK BETWEEN HER2/NEU AND BRCA1 IN SPORADIC BREAST CANCER

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The Breast Cancer Susceptibility Gene 1 (BRCA1) is frequently mutated in familial breast cancer. In contrast, in sporadic breast tumours, mutations in the BRCA1 gene are only very rarely found. Despite this, BRCA1 expression appears to be decreased in a large percentage of sporadic tumours, suggesting an important role for BRCA1 as a tumour suppressor in sporadic breast cancer as well. The phenotypic characteristics which describe sporadic breast tumours with particularly low BRCA1 levels, such as high tumour grade, increased metastatic potential and overall poor prognosis, are similar to those of tumours that overexpress the Human epidermal growth factor receptor 2 (Her2/neu). Several studies have shown a strong concordance between high Her2/neu levels and decreased BRCA1 expression in sporadic breast tumours. Our group previously identified the *ets*-related GA-binding protein (GABP) complex as a critical transcriptional activator of BRCA1 expression. GABP-dependent regulation of the BRCA1 promoter differs in MCF-7, T-47D and SK-BR-3 breast carcinoma cells. The activity of the GABP beta subunit in particular appears to be lost in SK-BR-3 cells, which highly overexpress Her2/neu and exhibit very low BRCA1 levels. Both GABP beta mRNA and protein levels are reduced in SK-BR-3 cells, suggesting that downregulation of GABP beta occurs at the transcriptional level. To investigate the transcriptional regulation of the GABP beta gene in the context of Her2/neu overexpression and loss of BRCA1, we have cloned the 800-bp GABP beta promoter region, and deletion constructs were created to further characterize the promoter. Transient transfections of the GABP beta promoter constructs show that GABP beta promoter activity is reduced in Her2/neu-overexpressing SK-BR-3 cells, while inhibition of Her2/neu by Herceptin increases GABP beta mRNA expression in these cells. These findings suggest a role for GABP as a potential link between overexpression of Her2/neu and loss of BRCA1 in sporadic breast cancer.

SaP51
SIRNA MEDIATED CHEMOSENSITIZATION OF BREAST CANCER CELLS BY INHIBITING AUTOPHAGY

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Autophagy, a process of "self-eating", is the innate response to starvation at the cellular level in organisms like yeast. In higher organisms, including humans, it has been shown to be involved in the processes of cell homeostasis by removing damaged or aging organelles and long-lived proteins. Recently, autophagy has also been linked to various pathologies including cancer. It has been hypothesized that autophagy can be either protective or destructive to cancer progression, depending on the particular type and stage of human cancer. For example, activation of autophagy might favor survival of cells during irradiation and thus promote tumor growth. Alternatively, activation of autophagy might lead to cell death and help eradicate tumor growth. Since the study of autophagy at the molecular level is a new and an emerging field, currently no viable methods exist which can directly quantify it in cancer treatment models. Therefore the initial aims of our project are 1) to establish a

system by which we can efficiently quantify and monitor autophagy and 2) study autophagy in different cancer cell models treated with current therapeutic agents. In order to achieve this, a FACS based strategy coupled with quantitative mRNA and protein analysis is employed. In addition, siRNA based knockdown is used to disrupt autophagy genes thereby allowing autophagy to be assessed selectively. Preliminary results indicate that a) the methodology devised has the potential to select and quantify autophagic positive cells in cancer treatment models and b) that autophagy might be cyto-protective as its inhibition in tamoxifen treated MCF7 breast carcinoma cells in culture leads to an increase in cell death. The work proposed here will help us evaluate the use of autophagy inhibition as a way to promote the effectiveness of current chemotherapeutic agents and irradiation.

SaP52
ACTIVATED AKT1 ACCELERATES MAMMARY TUMORIGENESIS IN THE MMTV-C-ERBB2 TRANSGENIC MICE WITHOUT ACTIVATION OF ERBB3
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ErbB2 is a member of the epidermal growth factor receptor (EGFR) family and is overexpressed in 20-30% of invasive human breast cancer cases. Expression of c-ErbB2 using the MMTV promoter results in mammary tumorigenesis. ErbB2 overexpression induces activation of Akt, a serine/threonine kinase. Activated Akt promotes cell survival, cell proliferation and alters metabolism. ErbB2-induced cellular transformation often depends on activation and overexpression of ErbB3, an EGFR family member and known ErbB2 heterodimerization partner. We have crossed the MMTV-Myr-Akt1 transgenic mice (which express constitutively active Akt1 in the mammary gland) with the line 202 MMTV-c-ErbB2 transgenic mice to evaluate the role of Akt1 activation in ErbB2-induced mammary carcinoma. Bitransgenic MMTV-c-ErbB2, MMTV-Myr-Akt1 mice develop mammary tumors twice as quickly as the MMTV-c-ErbB2 mice (110 days versus 231 days). Tumors derived from the MMTV-c-ErbB2 mice demonstrate overexpression of EGFR, ErbB2 and ErbB3 as well as phosphorylation of ErbB3 and Akt, underscoring the importance of the EGFR family in ErbB2-induced tumorigenesis. Tumors from the MMTV-c-ErbB2, MMTV-Myr-Akt1 bitransgenic mice demonstrate overexpression of the myr-Akt1 and ErbB2 transgenes, however there is significantly less overexpression and activation of ErbB3 and EGFR. This indicates that Akt1 activation alters the requirement for ErbB3 in ErbB2-induced tumors.
Magnetic resonance spectroscopy (MRS) analysis of tumors indicates that the two tumor types have different metabolic profiles with the bitransgenic tumors being more glycolytic. It remains to be determined whether this change is due to the Akt1 transgene or results from hypoxia in the rapidly growing tumor.

SaP53
HEREGULIN UPREGULATES MMP-9 ACTIVITY VIA AKT IN BREAST CANCER CELL LINES
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Several peptides such as epidermal growth factor (EGF), heregulin (HRG) and transforming growth factor alpha (TGFa) are ligands for EGFR family. Heregulin beta 1 (HRG-b1) binds to ErbB-3 and –4 and plays important roles in breast cancer cell proliferation and tumorigenesis. Here, we investigated the proteins through which HRG affects on MMP-9 activity. We treated HRG-b1 on breast cancer cell lines, SK-Br3, MCF-7 and MDA-MB-231. After 24 hrs, activity and expression levels of MMP-9 were increased but MMP-2 activity was not changed. The increasing rates of MMP-9 activity and expression were highest in SK-Br3 cell line. Upon HRG-b1 treatment, phosphorylation of Akt was also increased. In SK-Br3 cells, Akt phosphorylation showed peak in 30 min after treatment and after 6 hrs, the level was decreased. In MCF-7 cells, Akt phosphorylation showed highest level in 30 min after treatment and on 6 hrs, the level was decreased to 20% compared to the peak. But in MDA-MB-231 cells, Akt phosphorylation was merely increased. The expression levels of Akt were not changed upon HRG treatment. Phosphorylation of extracellular signal-regulated kinase 1/2 (ERK-1/2), down-stream molecules of Akt, was also increased by HRG treatment. Pretreatment of LY294002, PI3K inhibitor, almost completely blocked the MMP-9 activity. Our studies

suggested that Akt mediates the HRG signaling to MMP-9.

SaP54
ORGANOCHLORINE PESTICIDES INDUCE CHANGES IN INSULIN GROWTH FACTORS SIGNALING PATHWAY IN BREAST CANCER CELL LINES

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Hexachlorobenzene (HCB) is a widespread environmental pollutant. Chronic administration of HCB to experimental animals elicits hypothyroxinemia, and liver and thyroid carcinogenesis. We have previously demonstrated that HCB administration to rats decreased uterine estrogen receptor levels and reduced circulating estradiol, and enhanced the development and malignancy of NMU-induced mammary tumors in rats and altered insulin/IGF-I signaling pathway. The aim of this work is to compare the effects of HCB on: 1-cellular proliferation, 2-Isulin Receptor (IR), and Insulin Growth Factor–I Receptor (IGF-IR) protein contents and 3-Insulin Receptor Substrate-1 (IRS-1) protein levels and its phosphorylation in Estrogen Receptor (ER)-positive MCF-7 and ER-negative MDA-MB231 cell lines. The cells were grown in RPMI 1% antibiotic-antimicotic, glutamine and 10% FBS, and were exposed to HCB (0, 0.005, 0.05, 0.5 and 5 uM). For proliferation assays, cells were exposed for 7 days, and the number of colonies were counted. To evaluate receptor protein levels, cells were treated with HCB for 24 hours and cellular lysates were analysed by immunoblot. The results showed that in MCF-7 cells, HCB: a) enhanced cellular proliferation at 0.05uM, ($p<0.001$), b) decreased IGF-IR at 0.5uM,($p<0.01$), increased IR levels at 0.005 and 0.05uM,($p<0.05$) and decreased IRS-1 content at 0.005, 0.5 and 5uM,($p<0.05$). Its phosphorylation was augmented at 0.05uM,($p<0.05$). In MDA-MB-231cells, HCB: a) had no effect on proliferation, b) decreased IGF-IR at 0.005, 0.5 and 5uM,($p<0.05$), increased IR levels at 0.05uM,($p<0.05$) and increased IRS-1 content at 0.5 and 5uM. These results indicate different effects of HCB on the insulin/IGF-I signaling pathway in ER-positive and ER-negative breast cancer cell lines.

SaP55
LPA RECEPTORS IN BREAST CANCER CELL MOTILITY

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Lysophosphatidic Acid (LPA) acts via binding to specific G protein-coupled receptors and has been implicated in the biology of breast cancer. Here, we characterize LPA receptor patterns in common established breast cancer cell lines and their contribution to breast cancer cell motility. Measuring expression of LPA1, LPA2 and LPA3 by real-time quantitative PCR, we show that the breast cancer cell lines tested can be clustered into three main groups: cells that predominantly express LPA1 (BT-549, Hs578T, MDA-MB-157, MDA-MB-231 and T47D), cells that predominantly express LPA2 (BT-20, MCF7, MDA-MB-453 and MDA-MB-468) and a third group that shows comparable expression level of these two receptors (MDA-MB-175 and MDA-MB-435). LPA3 expression was detected primarily in MDA-MB-157 cells. Using a Transwell chemotaxis assay, we find that cells predominantly expressing LPA1 have a peak migration rate at 100nM LPA that drops off dramatically at 1μM LPA; while the cells predominantly expressing LPA2 show the peak migration rate at 1μM LPA which remains high at 10μM. Using BT-20 cells, LPA2-specific siRNA and C3 exotransferase, we demonstrate that LPA2 can mediate LPA-stimulated cell migration and activation of the small GTPase RhoA. Using LPA2 siRNA and expression of LPA1 in the BT-20 cells, we further find that LPA1 and LPA2 cooperate to promote LPA-stimulated chemotaxis. In summary, our results suggest that the expression of both LPA1 and LPA2 may have important implications for breast cancer biology by permitting cells to respond optimally to a wider range of LPA concentration, thus revealing a new aspect of LPA signaling in breast cancer.

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DIFFERENTIAL SRC AND FAK ACTIVATION CONTRIBUTES TO TGF-β; INDUCED MOTILITY AND INVASION OF NEU/ERBB-2 EXPRESSING BREAST CANCER CELLS

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Overexpression of the Neu/ErbB2 receptor tyrosine kinase is associated with breast cancer progression and correlates with poor patient prognosis. TGF-β is now recognized to play a dual role in many epithelial cancers, acting as a growth suppressor in normal cells and a tumor promoter in cancer cells. Cooperation between these two pathways enhances the invasive and metastatic abilities of breast cancer cells.

We have employed two different forms of an oncogenic Neu/ErbB2 receptor; one which possesses five intact tyrosine autophosphorylation sites within the C-terminus (Neu-NT) and a second containing tyrosine to phenylalanine mutations at each autophosphorylation site (Neu-NYPD). We have also employed an NMuMG mouse mammary epithelial cell model because these cells exhibit both a TGF-β-induced growth arrest and an epithelial-to-mesenchymal transition (EMT) that is correlated with enhanced motility and invasion. NMuMG populations (Neu-NT, Neu-NYPD or empty vector), were established after in vivo outgrowth in the mammary fatpads of athymic mice. The resulting tumors (Neu-NT and Neu-NYPD) were explanted into culture and found to retain overexpression of Neu/ErbB2, remain sensitive to TGF-β mediated growth inhibition and undergo a TGF-β induced EMT. NMuMG cells with the empty vector failed to form tumors, but could be explanted from residual cells at the injection site and served as negative controls in all assays. Transwell assays revealed a TGF-β induced increase in motility and invasion in the Neu-NT explants that was absent in cells expressing Neu-NYPD or harboring the empty vector control. This system has allowed us to begin to characterize those signaling pathways downstream of the Neu/ErbB-2 receptor that collaborate with TGF-β to promote breast cancer motility/invasion and identify key regulators involved in this process.