Design: Retinal pericytes were cultured from bovine retinas. These cultured cells were greater than 95% pure and only used from passages 3-6. Both palmitate and oleate were conjugated with bovine serum albumin at a 2:1 molar ratio. The cells were incubated in DMEM media with the indicated fatty acid and/or chemical agent for 24 hr and then tested for accumulation of ceramide mass, ER stress markers, NF-kappa B activity, and apoptosis (TUNEL and caspase assays).
Results: We discovered that activation of the stress activated kinase, AMP-activated protein kinase (AMPK), by the cell permeable AMPK activator AICAR (5-amino-4-imidazolecarboxamide riboside) or use of a constitutively active AMPK can prevent retinal pericytes from undergoing palmitate-induced cell death. Though the exact mechanism of palmitate-induced apoptosis is not currently known, we found that it may be brought on by several processes that cause cell death in the context of diabetes. We found that 1) ceramide mass was increased dose dependently by palmitate; 2) ER stress was upregulated as witnessed by a 2-4-fold increase in the ER stress markers BiP and CHOP at both the mRNA and protein levels; 3) NF-kappaB activity was increased as measured by reporter gene assay; and 4) that inhibition of any one of these processes was sufficient to rescue the BRPC from palmitate-induced cell death.
Conclusions: Activation of AMPK with AICAR was able to prevent all the processes studied herein, including apoptosis itself, therefore providing a potential therapeutic option for improving vascular homeostasis in diabetic retinopathy.

1525 Hypermethylation of the *p16^{INK4a}* Gene Promoter Seems To Be a Frequent Epigenetic Change in the Eyelid Sebaceous Carcinoma

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Background: Eyelid sebaceous carcinoma is an ocular malignancy with possible life-threatening consequences. Some Asian reports suggest that the human papillomavirus (HPV) may play a role in the development of this malignancy by disrupting the function of *p53*. Overexpression of p16^{INK4a} , which is also associated with the HPV's oncogenic mechanism, has also been reported in cases of eyelid sebaceous carcinoma. The authors wish to explore the molecular and epigenetic basis of HPV and the p16^{INK4a} status in the development of eyelid sebaceous carcinoma.
Design: Twenty-four cases of eyelid sebaceous carcinoma were analysed for the expression of p16^{INK4} via immunohistochemistry. Nested polymerase chain reaction (PCR) and genechip HPV typing were used to detect HPV infection and decide its genotype in our samples. The methylation status of *p16^{INK4a}* promoter region was studied by methylation-specific PCR.
Results: HPV-positivity was demonstrated in only one of our cases, while another case was immunoreactive for p16^{INK4}. *P16^{INK4a}* promoter hypermethylation was noted in nearly half of our cases (11/24) and was also associated with younger patient age (*p=0.013*).
Conclusions: Higher frequency of *p16^{INK4a}* promoter hypermethylation in eyelid sebaceous carcinoma may imply a significant epigenetic role in tumor development, more so than the presence of HPV.

1526 Pathological Review of 50 Intraorbital Meningiomas Using WHO 2007 Criteria

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Background: Meningiomas represent 2 to 4% of all intraorbital tumors. In clinical series, they are approximately equally divided between those arising from the optic nerve, and those extending into the orbit from adjacent structures, usually the sphenoid wing. Although the grading and subclassification of intracranial meningiomas has been relatively well studied, no large series of intraorbital meningioma has been reported using the grading scheme introduced by the WHO in 2000. Herein, we describe the histopathologic features and WHO grades of 50 intraorbital meningiomas.
Design: Intraorbital meningiomas diagnosed between 1968 and 2008 were retrieved from the archives of the Neuropathology and Eye Pathology Laboratories at our institution. Clinical data were also retrieved from the hospital archives, including patient age, sex, and intraorbital location of the tumor. Hematoxylin and eosin stained slides from all cases were reviewed according to the WHO 2007 classification scheme.
Results: A total of 50 intraorbital meningiomas were reviewed. The mean age at presentation was 45 years (range, 8 to 96 years), and four tumors arose in children, ages 8 to 15 years. Two patients were known to have neurofibromatosis type 2 (NF2). Intraorbital meningiomas were more frequently encountered in females (30 cases) than in males (20 cases). In 21 patients (13 females, 8 males), the tumor was associated with the optic nerve. In the remaining 29, the optic nerve was not known to be involved. The most common histopathologic subtype (28/50 tumors, 56%) was meningothelial. An additional 20 tumors (40%) were classified as transitional, and 2 (4%) were predominantly angiomatous. Focal microcystic change was present in 2 tumors. Most of the tumors (46/50, 92%) were WHO grade I, with less than 4 mitotic figures per 10 high power fields (hpf). Four tumors (8%) were WHO grade II (2 males and 2 females), with more than 4 mitotic figures per 10 hpf. One of these grade II tumor also exhibited brain invasion, and a second showed optic nerve invasion. Invasion of either dura, bone, muscle, orbital fat, lacrimal gland, choroid or sclera was observed in 19 cases.
Conclusions: In our series, intraorbital meningiomas were most frequently of the meningothelial or transitional subtypes, and WHO grade I. One relatively common intracranial subtype, fibrous meningioma, was not encountered. The percentage of WHO grade II tumors in the orbit (8%) is similar to that reported for intracranial tumors using the current grading scheme.

1527 Identification of IgG4 Positive Plasma Cells in Recurrent Idiopathic Orbital Inflammation but Not in Non-Recurrent Lesions

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Background: Idiopathic orbital inflammation (IOI) (inflammatory pseudotumor) is a rare entity that consists of chronic inflammatory cells with myofibroblasts in variable proportions that results in a variety of ocular manifestations including decreased and even loss of vision. IOI is typically steroid responsive but some require surgery. Recurrence is common; however, specific histopathologic features predictive of recurrence have not yet been identified. IgG4+ plasma cells have been detected in IOI and in some pseudotumors in various other locations, and high serum levels of IgG4 are associated with systemic syndromes. The purpose of this investigation was to evaluate the prevalence of IgG4 positive plasma cells in IOI and to identify its clinical significance.
Design: 25 cases diagnosed as IOI or orbital inflammatory pseudotumor from 1988 to 2008 comprising 16 patients were retrieved from departmental files. Sections were cut and stained with a mouse anti-human monoclonal antibody to IgG4. Normal human tonsils were used as positive control. If any cells with cytoplasmic/plasma membrane reactivity for IgG4 in10 high powered fields (HPF; 40X) were found, the specimen was considered positive for IgG4. Statistics were performed using a Fisher two tailed t test.
Results: Among the 25 unique biopsies with a histological diagnosis of IOI, 16 (in 10 out of 16 patients) had IgG4+ plasma cells. Of these 10 patients with an IgG4+ biopsy at any time during their series, 6 patients had recurrent lesions (sensitivity=100%, specificity=60%, p=0.03). None of the IgG4 negative cases recurred (negative predictive value (NPV)=1, p=0.03, 95% CI= 0.52 to 1). A positive predictive value of 0.73 was obtained when the threshold was raised to ≥ 20 IgG4+ cells/10 HPF (p=0.04). Only one patient with recurrent IOI presented with an IgG4 negative biopsy on the first specimen (sensitivity=83%, specificity=60%, NPV=0.86, p=0.15).
Conclusions: The absence of IgG4+ plasma cells is a strong predictor of the lack of future recurrence of IOI requiring surgical treatment. This finding may have potential implications for clinical diagnostics and pathophysiology of IOI.

1528 Autotaxin Expression in Ocular Tissues and Uveal Melanoma

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Background: Uveal melanoma is the most common primary intraocular malignancy. In spite of advancements in diagnosis and treatment, mortality rates have not improved. Efforts to stratify patients for possible systemic treatment have looked at various histopathologic and molecular features, including monosomy 3. Microarray gene profiling has revealed two classes: class 1 (low grade) and class 2 (high grade), the latter more predictive of liver metastasis. In a recent study, gene expression profiling also demonstrated that underexpression of an enzyme called autotaxin could predict the class 2 tumors. It has been found to be upregulated in some other malignancies, such as breast and prostate carcinoma. Autotaxin expression in the eye and in particular in uveal melanoma has not previously been described.
Design: This study undertook to assess the staining patterns of this enzyme by immunoperoxidase in 15 eyes with uveal melanomas treated by enucleation. Stains were performed alone (12 cases) or as a double stain with a macrophage marker (CD68) along with autotaxin (3 cases). The results were correlated with FISH results for monosomy 3 (available in 14 of the 15 cases).
Results: Positive staining for autotaxin (positive control: prostatic adenocarcinoma) consisted of a cytoplasmic blush and/or punctate cytoplasmic granules. Strong positivity was found uniformly in all layers of the retina in all 15 eyes. The non-pigmented ciliary epithelial cells, retinal pigment epithelial cells, corneal epithelium and smooth muscle of the ciliary body were also variably positive. The corneal stroma and sclera were uniformly negative. Of the 15 cases of uveal melanoma, 3 were completely negative (0% of cells) for autotaxin. In another case, there was scant staining (5% of cells). Of these 4 cases, 3 showed monosomy 3 by FISH (no FISH results for fourth). In 11 cases, there was staining for autotaxin in variably 10% to 100% of cells. Of these 11 cases, only 3 had monosomy 3. Staining was variable within the tumors, presumably due to variable fixation.
Conclusions: 1) Autotaxin is overexpressed in some malignancies but appears to be downregulated in uveal melanomas. 2) Immunoperoxidase staining for autotaxin may offer an economical way to stratify patients for aggressive treatment in an effort to prevent liver metastasis. 3) Additional work is needed to further classify the patterns of staining of this antibody, as experience is so far limited.

Pathobiology

1529 Cancer-Promoting and Initiating Stem Cells Can Be Derived from Ectopic Locations in Breast Cancer

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Background: Although human breast cancer is all too common, circumstantial evidence exists to suggest that cancer transformation is a rare event. Even in the setting of inherited breast cancer, eg. BRCA1 when all the cells of the breast contain the inherited BRCA1 mutations, transformation on a cellular level is still rare. This suggests that only certain cells are capable of cancer initiation and promotion. Cancer-promoting and cancer-initiating stem cells, while mainly residing in the organ of cancer origin, can also be derived from ectopic locations. In a previous study of human transplant recipients who had received sex-mismatched bone marrow and other organ transplants for various diseases and later developed secondary solid cancers including breast cancer, cancer-promoting stem cells of donor origin giving rise to lymphocytes, fibroblasts,

myofibroblasts, tissue macrophages and endothelial cells and rarely, cancer-initiating stem cells were observed within the secondary solid cancer.
Design: Carrying forward these observational human studies to testing these hypotheses experimentally in mouse models, we conducted bone marrow transplantations in transgenic mice genetically engineered to develop breast cancer. Being able to mark the donor bone marrow enzymatically, we were able to study the breast cancers that developed in the mammary gland for the presence of stem cells of donor origin. Using either the bitransgenic *MMTV-pymT/ROSA26* or the *MMTV-erbb2/neu/ROSA26* models as donors and either sublethally irradiated wild type or single unmarked transgenics as recipients, we investigated the donor v recipient origin of the cancer cells and the cells of the tumor microenvironment.
Results: With either bitransgenic model as donor, we were able to demonstrate that the breast cancers that emerged contained significant percentages of tissue macrophages, lymphocytes, fibroblasts, myofibroblasts and endothelial cells, which represented the progeny of cancer-promoting stem cells of donor origin. These cells were present at very early stages of tumor progression. Rare cancer-initiating stem cells of donor origin were also observed.
Conclusions: These ectopically-derived cells of bone marrow origin present within the tumoral microenvironment may affect breast cancer progression differently from those of endogenous orign and may further contribute to the heterogeneity of the tumor microenvironment. The rare cancer-initiating stem cells of donor origin are additional proof that stem cells can initiate breast cancer.

1530 The Adenosinergic A2A Receptor Prevents Exacerbation of Systemic Inflammation and Lung Inflammation Secondary to Polymicrobial Sepsis

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Background: Endogenous tissue protection mechanisms protect normal tissues from immune cell mediated damage during inflammation. One pathway responsible for the inhibition of activated immune cells is the hypoxia-driven adenosine A2A receptor (R) mediated pathway. A2AR activation in direct inhalation models of acute lung injury prevents immune cell mediated destruction of normal tissues. However, it is unclear whether A2AR's protect pulmonary tissue during systemic inflammation.
Design: Male C57BL/6 mice and A2AR knock out (KO) mice were subjected to cecal ligation and puncture (CLP)-induce sepsis and assessed for systemic inflammation 6 hours post-CLP and lung injury at 24 hours.
Results: Systemic inflammation is exacerbated in A2AR KO mice compared to wt mice. A2AR KO mice had significantly increased serum IL-6 levels vs. wt mice (Table; P<0.001). To determine the effect of A2AR signaling on lung inflammation secondary to systemic infection, bronchoalveolar lavage fluid (BALF) was analyzed for total protein content and total cell count following CLP. Both cell number and total protein were increased in BALF of A2AR KO CLP mice compared to wt CLP mice (Table; P=0.026, P=0.0098, respectively).

A2AR KO Mice Have Increased Inflammation During Sepsis.

CLP group	*IL-6 (pg/ml)	*BALF total cell count	*BALF protein (ug/ml)
Wild type	8,371 ± 1,646	36,710 ± 5,944	108.2 ± 11.45
A2AR KO	18,110 ± 2,041	63,750 ± 7,603	157.2 ± 9.708

All groups are expressed as mean +/- SE; n=6-14. *Statistical significance is defined as P<0.05.
Conclusions: Increased systemic inflammation with increased secondary lung inflammation was found in the absence of A2AR signaling during sepsis. These findings suggest that adenosinergic A2AR's function to inhibit exacerbation of pulmonary inflammation during sepsis.

1531 Is There a Clinical or Diagnostic Utility for *BRAF* V600E Mutation in Thyroid Lesions?

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Background: Thyroid carcinoma develops as a consequence of accumulation of molecular genetic changes in the follicular cells, characteristically involving RET/PTC and *B-RAF* mutations. Although the V600E point mutation in the *BRAF* oncogene was reported in papillary thyroid carcinoma (PTC), the sensitivity and specificity of the *BRAF* mutation in various thyroid carcinomas have not been fully investigated. In addition, if the *BRAF* mutation is sensitive and specific for PTC, it could be a helpful tool in diagnosing difficult cases.
Design: Sixty four cases of resected thyroid lesions included papillary carcinoma (34), follicular adenoma (n=10), follicular carcinoma (n=6), Hashimotos thyroiditis (6), medullary carcinoma (4), C-cell hyperplasia (1), nodular hyperplasia/goiter (2), and lymphoma (1) were examined for this study. The lesions were microdissceted from the surrounding non-lesional thyroid tissue; total genomic DNA was purified and amplified using specific primers that flanked the *BRAF* mutation. The PCR product was purified and subsequently sequenced in both directions using the same primer set and an ABI 3100-Avant system to identify the codon mutation at amino acid 600 of the *BRAF* gene.
Results: Eleven out of 34 (32.4%) PTC contained the V600E *BRAF* mutation, including 7 conventional PTC, 2 follicular variant PTC and 2 papillary microcarcinomas. The mutation was only observed in the carcinoma cells; not in the surrounding normal tissue. The mutation was not associated with gender, age, or the size of the lesion. The average tumor size was 2.1 ± 1.2 cm in the mutant group and 1.5 ± 1.2 cm in the wild type (WT) group (p=0.233). The average age was 51.5 ± 15.8 (range 32 to 77) years and 48.5 ± 10.8 (range 26 to 67) years in the mutant and WT groups, respectively (p=0.57). Both mutant and WT groups had very few cases with cervical lymph node metastasis for meaningful evaluation. No other lesion harbored the mutation at the V600E locus of the *BRAF* gene.
Conclusions: The V600E mutation of the *BRAF* gene is specifically involved in the carcinogenesis of PTC (100% specificity) but was detected in a minority of the cases (32.4% sensitivity). The *BRAFV600E* mutation is not associated with the tumor size and patients' age and sex, therefore limiting its usefulness in prognosis of PTC. No *BRAF* mutation was detected in any other lesions which may be helpful in differential diagnoses of an atypical thyroid aspiration in a setting of thyroid nodular lesions.

1532 Reverse Phase Protein Lysate Array (RPPA): Use in Identifying Function for Estrogen-Induced Gene 121 (EIG121)

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Background: RPPA is a high-throughput "dot blot" in which dilutions of protein lysate are spotted on nitrocellulose slides. Each slide is probed with a different monospecific antibody. A DAKO-catalyzed signal amplification system is used for signal detection. Each slide holds a maximum of 1,152 spots, thus making the assay amenable to high-throughput analysis. An advantage of RPPA over Western blots is that multiple replicates and dilutions can be incorporated into the experimental design, thus making protein level quantification more accurate. An advantage over immunohistochemistry is that RPPA is quantitative rather than qualitative. We used RPPA to help characterize the function of EIG121, a gene we discovered from a microarray analysis of baseline and post-treatment endometrial biopsies from women taking estrogen-based hormone replacement therapy. EIG121 is over-expressed in estrogen-dependent endometrioid-type endometrial carcinoma, but not non-endometrioid tumors. Other than being a lysosomal/endosomal protein, little is known of EIG121's function.
Design: Protein lysates were prepared from 200 endometrial carcinomas and spotted on RPPA slides. Antibodies against 82 different proteins were applied to the RPPA slides. These antibodies were previously validated by Western. Antibodies were directed against receptor tyrosine kinases, members of the PI3K/AKT, JAK-STAT, and MAPK pathways, and proteins regulating apoptosis and the cell cycle. Correlation coefficients were calculated to identify proteins that were associated with EIG121 expression.
Results: EGFR and HER-2/neu were 2 of the proteins with highest negative correlations with EIG121 expression (i.e., tumors with high EIG121 expression had lowest expression of EGFR and HER-2/neu). Evidence in breast cancer literature suggests that endocrine sensitivity is inversely related to growth factor-mediated signaling. We speculated that lysosomal/endosomal EIG121 binds to and degrades growth factor receptors. In cell-based systems, we have subsequently shown by co-IP that EIG121 binds to EGFR, promotes its degradation, and inhibits downstream signaling via Akt.
Conclusions: RPPA provided critical clues that helped us to identify EIG121 as an important "molecular switch" that regulates the hormone responsiveness/resistance of tumor cells. We speculate that this "switch" function of EIG121 may be crucial in dictating an endometrial tumor's histotype (endometrioid vs. non-endometrioid).

1533 Blocking Lipocalin 2 Function Impairs Mammary Tumor Formation and Lung Metastases

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Background: Breast cancer is curable in its early stage. Most cancer deaths are caused by advanced disease and distal metastases. Therefore, identifying and targeting the molecules involved in tumor progression and metastases are the key to reducing breast cancer mortality. In the current study, we identify lipocalin 2 (LCN2) as a marker of poor prognosis, demonstrate its role in tumor cell migration, invasion and metastasis *in vitro* and *in vivo* in mouse models.
Design: 318 primary breast cancer samples were analysed by Affymetrix microarray; LCN2 expression was correlated with clinical and pathological features. Xenograft models with human breast cancer cell lines with LCN2 overexpression and knockdown were established; MMTV-HER2 (V664E) transgenic mice were bred with LCN2 knockout mice to evaluate the function of LCN2 *in vivo*. Finally, anti-LCN2 antibody was generated and therapeutically injected into mammary tumor-bearing mice attempting to block lung metastases.
Results: Microarray analysis of 318 patient samples demonstrated that LCN2 is associated with adverse clinicopathological features (Table 1). The role of LCN2 in tumor progression was further illustrated in the xenograft mouse model and MMTV-HER2 transgenic LCN2 null mice. Human breast cancer cell lines with enforced expression of LCN2 increased tumorogenicity in nude mice. In contract, knockdown LCN2 in high expressing breast cancer cell lines reduced tumor invasion and metastases *in vivo* in nude mice. Deficiency of LCN2 in MMTV-HER2 transgenic mice led to impared mammary tumor formation and reduction in lung metastases. Furthermore, injecting anti-LCN2 neutralizing antibody through the tail vein significantly blocked lung metastases in mammary tumor-bearing mice.
Conclusions: LCN2 is a marker of poor prognosis in breast cancer. It plays critical roles in promoting tumor progression and metastasis. Blocking LCN2 function can block lung metastases.

Table 1. High LCN2 expression is associated with adverse clinicopathologic features.

Clinicopathologic Features	**Statistical Significance**
High expression vs. low expression	
ER - vs. ER +	$p < 0.001$
HER2 + vs. HER2 -	$p < 0.05$
Tumor stage 3 and 4 vs. 1 and 2	$p < 0.02$
Nuclear grade 3 vs. 1 and 2	$p < 0.005$
Axillary lymph node metastasis vs. non-metastasis	$p < 0.01$

The statistical significance was identified in three analyses including Spearman's rank correlation, unequal variance t-test and Wilcox rank sum test.

1534 Gossypol Suppresses In Vitro Head and Neck Squamous Cell Carcinoma Growth through Its Inhibition of DNA Methyltransferase 1 and Epigenetic Activation of Tumor Suppressor Genes

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Background: Most forms of human malignancy display frequent epigenetic alterations in association with promoter hypermethylation. A key enzyme involved in the initiation and maintenance of DNA hypermethylated state is DNA methyltransferase (DNMT) 1 and this gene is overexpressed in many forms of human malignancies. Gossypol, a male antifertility agent and a natural polyphenolic compound present in cottonseeds, has been shown to possess antiproliferative and pro-apoptotic effects both in vivo and in vitro in a variety of human tumors through its potent inhibition of Bcl2 family of antiapoptotic proteins, protein kinase C, and Cyclin D1. Here, we reported that gossypol is a novel small molecule inhibitor of DNA methyltransferase 1 and can suppress in vitro head and neck cancer growth through its epigenetic effects.

Design: In vitro methylation assay was used to evaluate the inhibition of DNMT1 by gossypol. DNA pyrosequencing and methylation-specific PCR were used to analyze changes in DNA methylation. Real-time RT-PCR and western blot analysis were used to characterize expression of tumor suppressor genes. For in vitro tumor suppression, 6 HNSCC cell lines (UMSCC1, SQ-20B, T-167, T409, TU167, and MDA1986) and a normal oral keratinocyte cell line (HOK-16B) were treated with gossypol followed by determination of cell viability.

Results: Gossypol showed dose-dependent inhibition of DNMT1 in in vitro cell-free methylation assay. As a result, gossypol significantly reduces DNA methylation in the promoter region of MGMT and E-cadherin genes, resulting in reactivation of these 2 tumor suppressor genes. Gossypol also showed dose-dependent inhibition of 5 out of 6 (80%) HNSCC cell lines (UMSCC1, SQ-20B, T-167, T409, and TU167) at biologically achievable doses (less than 10 μM) with an IC50 ranging from 2.8 μM to 6.2 μM for these 5 cell lines.

Conclusions: Gossypol is a novel and potent small molecule inhibitor of DNMT1 with effective DNA demethylating property. In HNSCC, gossypol can suppress in vitro tumor growth in part through epigenetic activation of tumor suppressor genes. Therefore, gossypol.

1535 Effect of DNA Hypomethylation on Lung Carcinogenesis

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Background: Lung cancer is the leading cause of cancer-related deaths in both sexes. Aberrant DNA methylation is one of the most common molecular lesions in pathogenesis of lung carcinoma. Enzymes responsible for this methylation are DNA methyltransferases (*Dnmts*). It has been previously shown that global DNA hypomethylation induced by hypomorphic *Dnmt1* alleles suppresses intestinal tumorigenesis in *Apc* Min/+ mice but promotes T cell lymphoma and sarcoma in mice. Its effect in lung carcinogenesis is not clearly elucidated. The main objective of this project is to study the effect of DNA hypomethylation on lung tumorigenesis in mice.

Design: Different mutant alleles of *Dnmt1* were used for this study (*chip/c; chip/chip; dnmt1* $^{2lox/2lox}$) with *c* being the null allele and *chip* providing 10% of the methylation. The study was done in a background of *LSL-K-ras* ^{G12D} conditional mouse model for mutant *K-ras* developed in Jacks' Lab. *LSL-K-ras* ^{G12D} mice were crossed to *Dnmt1* $^{chip/chip}$ and *Dnmt1* $^{chip/c}$; and *LSL-K-ras*^{G12D}*,Dnmt1* $^{chip/chip}$ double mutants were crossed to conditional *Dnmt1* $^{2lox/2lox}$ mice to obtain various combinations of double mutants. Mice were then infected with AdenoCre intranasally to initiate tumorigenesis via mutational activation of *K-ras* which is regulated by a removable *STOP* element flanked by *LoxP* recognition sites for recombinase Cre. Mice were sacrificed (n=125) at different time points and their lungs dissected. H and E sections were examined and number of lesions, which included atypical adenomatous hyperplasia, adenoma and adenocarcinoma, was counted for each of the *LSL-K-ras* ^{G12D} mice (positive controls) and compared to the experimental hypomethylated mice.

Results: Ratio of average number of lesions in *LSL-K-ras* ^{G12D} versus hypomethylated mice at different time-points is as follows: 2 weeks =1.85; 4 weeks = 1.9; 12 wks = 2.5; 16 weeks = 2.45; 28 weeks = 4.

Conclusions: The data shows that the number of lesions formed in *LSL-K-ras* ^{G12D} mice is about 2 to 4 times more than in the hypomethylated mice and that genomic hypomethylation induced by the mutant alleles of *Dnmt1* markedly lowers the number of lung tumors. This may have therapeutic implications in terms of use of demethylating agents in the treatment of lung cancers.

1536 Regulation of Rad18 Via the Ataxia Telangiectasia and Rad3 Related (ATR) Kinase

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Background: Carcinogenic DNA-damaging agents induce DNA lesions termed bulky adducts. During DNA replication, DNA polymerases encountering such adducts are stalled. Translesion synthesis (TLS) is a DNA repair mechanism by which, specialized DNA polymerases are recruited to stalled replication forks and perform DNA synthesis across damaged sites. Recruitment of TLS polymerases to stalled replication forks requires an E3 ubiquitin ligase RAD18. RAD18 functions by mono-ubiquitinating the polymerase processivity factor PCNA. PCNA interacts directly with TLS polymerases and acts as a polymerase switch, which enables cells to preserve replication forks that encounter DNA damage. Cells lacking RAD18 or TLS polymerases are highly sensitive to DNA damage-induced lethality. However, the mechanism(s) by which RAD18 senses DNA damage and gets recruited to the site of lesion are unclear. We and others have hypothesized that DNA damage-induced checkpoint signaling may promote TLS. Checkpoint kinases such as ataxia telangiectasia mutated and Rad3-related (ATR) have been previously reported to play a role in PCNA ubiquitination. A recent proteomic screen identified Ser 403 of RAD18 as a potential substrate for the checkpoint kinases ATM and ATR (Matsuoka et. al. Science 07). Therefore, ATM/ATR-mediated phosphorylation of RAD18 provides a potential mechanism for regulation of TLS by checkpoint signaling.

Design: To test our hypothesis that RAD18 is phosphorylated at S403 in response DNA damage we made a phosphorylation mutant (S403-A). Both ATM and ATR were identified as potential kinases for RAD18, therefore, we tested the hypothesis that checkpoint signaling regulates RAD18 in response damage. Also we tested whether S403 phosphorylation regulates RAD18-dependent TLS. This study provides new insights into the mechanisms by which integration of checkpoint signaling and TLS helps maintain genomic stability and prevent cancer.

Results: Our preliminary results suggest that Rad18 is phosphorylated basally at S403 site. Intrestingly, the phosphorylation at this site increases upon DNA damage. Our data also indicates that the phosphorylation at this site is required for successful cell cycle progression. Absence of phosphorylation (as tested using a S403 to A mutant) leads to accumulation of cells in the S phase. Failure to phosphorylate Rad18 at S403 also leads to upregulation of DNA damage markers such as P-Chk1 and P-H2Ax.

Conclusions: Rad18 S403 site seems to be phosphorylated upon DNA damage. This phosphorylation is required for cell cycle progression and viability upon DNA damage.

1537 NADPH Oxidase NOX5-S Mediates Acid-Induced Increase in H_2O_2 Production and Cell Proliferation in Barrett's Esophageal Adenocarcinoma Cells

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Background: Gastro-esophageal reflux disease complicated by Barrett's esophagus (BE) is a major risk factor for esophageal adenocarcinoma (EA). The mechanisms whereby acid reflux may accelerate the progression from BE to EA are not fully understood. We therefore investigated the role of NADPH oxidases in acid-induced changes in Barrett's EA cell line FLO.

Design: FLO cells or BE mucosal biopsies were exposed to pH 4.0 for 1hr, then washed and cultured at pH 7.2 for 24hrs. The mRNA levels of NOX5-S were measured by real time PCR. Cell proliferation was determined by measuring thymidine incorporation. Rho kinase activity was determined by a Cyclex ROCK assay kit.

Results: RT-PCR and 5'-RACE showed that NOX5-S was the major isoform of NADPH oxidase in FLO cells. NOX5-S mRNA expression was significantly greater in EA cell lines (FLO, and OE33) than in esophageal squamous epithelial cell line HET-1A. The levels of NOX5 mRNA were also markedly increased in BE mucosal biopsies with moderate dysplasia and in EA tissues, when compared with normal esophageal mucosa or BE mucosa. Immunohistochemical studies showed that NOX5-S was present in the cytosol of FLO cells. In FLO-EA cells, knockdown of NOX5-S with NOX5 siRNA significantly decreased cell proliferation at basal condition and inhibited acid-induced increase in cell proliferation. Acid treatment significantly increased H_2O_2 production in both FLO cells and BE mucosa. Acid-induced H_2O_2 production was blocked by the NADPH oxidase inhibitor apocynin. Acid increased mRNA expression of NOX5-S, and knockdown of NOX5-S abolished acid-induced H_2O_2 production in FLO cells. Acid-induced NOX5-S expression and H_2O_2 production were significantly decreased by MAP kinase kinase inhibitor PD98059 and Rho kinase inhibitor Y27632. In addition, acid treatment significantly increased the activity of Rho kinase and the phosphorylation of ERK2 MAP kinase. Acid-induced increase in phosphorylation of ERK2 MAP kinase was abolished by Y27632.

Conclusions: In EA cells acid induces H_2O_2 production by activation of NOX5-S and causes upregulation of NOX5-S expression through sequential activation of Rho kinase and ERK2 MAP kinase. In these cells NOX5-S contributes to increased cell proliferation.

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1538 Elucidating Signaling Networks in Clinical Tissues with Multispectral Imaging

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Background: A common goal in clinical research is revealing correlations between outcomes and complex protein expression patterns in tissue sections. Correlations inform target validation, trial design, patient selection, response assessment, and, if trials are successful, the diagnostic component of theranostics. However, to successfully detect multiple, often weakly-expressed targets in clinical tissue sections requires appropriate staining protocols, advanced instrumentation and powerful software. After developing multi-label immunohistochemical staining methods that were quantitative, independent, and specific, the goal was to create and validate an automated, whole-slide scanning imaging system to capture and distinguish multiple labels. Such a system would have an immediate application to signal-transduction research applied to conventional tissue sections. Here we describe this platform, and present results obtained from analysis of cancer tissue microarrays (TMAs).

Design: Multispectral imaging, using a spectrally enabled whole-slide scanning system, was performed on two triple-stained TMAs: the first stained with QDots targeting pMEK and pAKT; and the second targeting p53 and stathmin, both counterstained with a Dapi. Immunofluorescence (IF) signals were spectrally unmixed and isolated from each other. Image analysis algorithms were used to differentiate relevant tissue regions (e.g., malignant and normal epithelia, stroma, necrosis, etc.) and segment cellular compartments (nuclei, cytoplasm, and membrane) to extract IF signals on a per-cell basis. Per-cell relative stain intensities were analyzed with flow-cytometry analysis software.

Results: Multispectral 20x images obtained of each TMA core were acquired and spectrally unmixed at a rate of three cores per minute. Automated image analysis, using algorithms developed by end-users in under 1hour, took 10 seconds per core, segmenting cancer-containing regions and extracting signals from relevant cell compartments. Protein expression levels resulted in relatively weak but specific QDot signals, being 10-fold lower than the nuclear label, and 2-fold lower than tissue autofluorescence.
Conclusions: Multiplexed staining and detection, coupled with flow-cytometry analysis tools can reveal multiple protein expression patterns on a cell-by-cell basis, not possible with serial single stains. The innovative multispectral platform and software can capture cellular and subcellular expression details in an intact tissue architectural context.

1539 Topographic Relationship of High-Grade PIN with Atrophy and Adenocarcinoma

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Background: Atrophy of the prostatic secretory epithelium is ubiquitous in the adult prostate. Controversy has arisen regarding the putative relationship between atrophy, high-grade prostatic intraepithelial neoplasia (HGPIN), and adenocarcinoma.
Design: The study group consisted of 42 fully-embedded whole-mounted radical prostatectomy specimens from untreated patients. The percentage of each case involved with atrophy was estimated. Foci of HGPIN and adenocarcinoma were demarcated, the zonal location(s) recorded, and volume and pattern of HGPIN and cancer determined. HGPIN or adenocarcinoma was defined as *merging* when in physical contact directly with atrophy within an acinus, *adjacent* when HGPIN or cancer and atrophy abutted, *near* when occurring within 1 mm, and *distant* when occurring at a distance of more than 1 mm.
Results: The mean area involved by atrophy was 30.5% (range 5%-70%). A total of 375 foci of HGPIN were observed; 356 and 19 foci occurred in the peripheral and transition zones, respectively. Predominant patterns of HGPIN included 233 tufted (62.1%), 101 micropapillary (26.9%), 37 flat (9.9%), and 4 cribriform (1.1%). The mean volume of HGPIN was 0.022 cm^3 (range 0.003 – 0.4 cm^3). Eighty foci (21.3%) of HGPIN merged with atrophy, 24 foci (6.4%) were adjacent to atrophy, 189 (50.4%) foci were near atrophy (within 1 mm), and 82 foci (21.9%) were distant from atrophy. Six of these foci occurred where HGPIN was seen merging with atrophy; 1 focus occurred in atrophy distant from HGPIN or cancer. There was no association between the HGPIN volume and the extent of atrophy (p=0.09), and no association between the pattern of HGPIN and nearest atrophy (P=0.08). Sixty foci (16%) of HGPIN were adjacent to cancer, 76 foci (20.3%) of HGPIN were near cancer, and 239 (63.7%) foci of HGPIN were distant from cancer. Of the foci of adenocarcinoma nearest atrophy, 354 (94.4%) and 21 (5.6%) foci occurred in the peripheral and transition zones, respectively. The mean volume of HGPIN was significantly greater (0.035 cm^3, p value 0.0004) when it occurred adjacent to cancer than near or distant to cancer.
Conclusions: High-grade prostatic intraepithelial neoplasia occurred most commonly less than 1 mm from atrophy (50.4%), but the widespread universal presence of atrophy limits the interpretation of the significance of this finding. HGPIN volume was not associated with the atrophy volume. High-grade prostatic intraepithelial neoplasia had significantly greater volume when it occurred adjacent to cancer.

1540 Epidermal Growth Factor Receptor Abnormalities Are Frequent in Thymoma and Correlate with Clinico-Pathological Characteristics

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Background: In general, thymomas are indolent tumors with a tendency towards local recurrence, and occasionally, metastasis. The molecular pathogenesis of thymomas is starting to be delineated. Epidermal growth factor receptor (EGFR) abnormalities are frequent in epithelial tumors, and they have not fully characterized in thymomas.
Design: We investigated the frequency of EGFR abnormalities in a large series (N=115) of surgically resected thymomas, and correlated them with clinico-pathological features, including Suster and Moran (S-M) and WHO pathological classifications, and Masaoka's staging system. Using immunohistochemistry (IHC), total and phosphorylated (p)-EGFR (cytoplasm/membrane), TGF-a (cytoplasm), Ki67 and p53 (nuclear) were examined in all tumors. IHC expression was assessed using semiquantitative scores. *EGFR* gene copy number was studied in a subset of 67 cases using fluorescent in situ hybridization (FISH).
Results: High levels of EGFR (membrane score≥200=54/114, 47%), p-EGFR (membrane score≥50=16/115, 14%) and TGF-a (cytoplasmic score≥200=49/111, 44%) protein expression were detected in thymomas, especially in tumors with more advanced clinico-pathological features. *EGFR* FISH abnormalities were frequently detected in the 67 thymomas examined: disomy 21 (31%), trisomy 29 (43%), low polysomy (LP) 14 (21%), and high polysomy (HP) 3 (5%). No true *EGFR* gene amplification was detected. Higher levels of *EGFR* copy number changes were detected in more advanced thymomas based on pathological classification and stage. The highest levels of gene polysomy were detected in S-M atypical thymoma (LP 35% and HP 18%), WHO B2 and B3 thymomas (LP 40% and HP 10%), and stage IV tumors (LP 73% and HP 100%). Higher IHC expression of membrane EGFR and p-EGFR, cytoplasmic TGF-a and nuclear p53 was detected in cases having higher levels of *EGFR* gene copy number abnormalities.
Conclusions: Our findings indicate that EGFR gene copy number gain and protein overexpression are relatively frequent in thymomas, and correlate with higher malignant potential and more advance pathological stage. These findings suggest that EGFR abnormalities play an important role in the pathogenesis of thymomas, and EGFR inhibitors may be considered in the development of targeted therapy for this neoplasm. (Supported in part by the Thymoma Foundation).

1541 Correlated Alterations in Prostate Basal Cell Layer and Basement Membrane

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Background: It has ben speculated that focal basal cell disruption (FBCLD) induced auto-immunoreactions represent a contributing factor for human prostate tumor progression and invasion (Man and Gardner. Med Hypoth 70: 387-408, 2008; Man and Gardner. Intern J Biol Sci 246-258,2008). Since the basement membrane surrounds and attaches to the basal cell layer, our current study assessed whether FBCLD would impact the physical integrity of the associated basement membrane.
Design: Paraffin sections from 25-human prostate tumors with both pre-invasive and invasive components were subjected to immunohistochemistry with an innovative double immunostaining protocol, to simultaneously elucidate the basal cell layer with basal cell phenotypic markers cytokeratin (CK) 34ßE12 and p63, and the basement membrane with corresponding markers collagen IV and laminins. The physical integrity of the basement membrane near FBCLD (defined as the absence of basal cells resulting in a gap greater than the combined size of at least three basal cells) was examined to determine the extent of correlated alterations.
Results: The frequency of FBCLD varied significantly among cases. Although most FBCLD were seen in prostatic intraepithelial neoplasia (PIN), about 30% of FBCLD were seen in hyperplastic or normal appearing ducts or acini. Of a total of 89 FBCLD detected, 76 (85 %) showed correlated alterations in the overlying basement membrane, which included distinct focal disruption or fragmentation. The basement membrane overlying the remaining 13 (15%) FBCLD showed varying degrees of attenuation or reduction of the immunostaining intensity, compared to its adjacent counterpart overlying the non-disrupted basal cell layer. Focal disruptions in both the basal cell layer and basement membrane generally occurred near morphologically distinct basal cells that lacked expression of tumor suppressor p63.
Conclusions: These findings suggest that focal disruptions in the basal cell layer and alterations in the basement membrane may be a correlated event, and that basal cells may contribute to the production of the basement membrane ((Supported by in part by grants DAMD17-01-1-0129, DAMD17-01-1-0130, PC051308 from Congressionally Directed Medical Research Programs, BCTR0706983 from The Susan G. Komen Breast Cancer Foundation to Dr. Yan-gao Man, and 2006CB910505 from the Ministry of Chinese Science and Technology Department to Drs. Xichen Zhang, Yan-gao Man, and Guiyuan Li).

1542 Expression of IMP3, an Oncofetal Protein, in Various Malignant Primary and Metastatic Neoplasms, a Study of 1099 Cases

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Background: IMP3, an oncofetal protein, plays an important role in tumor proliferation and invasion. Recently, we have demonstrated that IMP3 is a new prognostic biomarker for cancer progression and metastasis of renal cell carcinoma and urothelial carcinoma. However, little is known about expression of IMP3 in other malignancies. In this study, we investigated expression of IMP3 in 1099 cases of various primary and metastatic carcinomas.
Design: 907 primary (P) malignant tumors and 192 metastatic (M) carcinomas selected from the surgical pathology files of the Departments of Pathology of the University of Massachusetts Medical Center were examined by immunohistochemical analysis. These cases included urothelial carcinoma (UC, P=238; M=28); skin melanoma (SM, P=78; M=11) endometrioid carcinomas of uterus (EMU, P=70; M=6); papillary thyroid carcinoma (PTC, P=21; M=10); squamous cell carcinoma of the skin (SCC, P=10; M=5); renal cell carcinoma (RCC, P=159; M=36); ductal carcinoma of the breast (DCB, P=24; M=27); colonic adenocarcinoma (CA, P=86; M=30); non-small cell carcinoma of the lung (NSCCL, P=26; M=10); prostatic adenocarcinoma (PA, P=35; M=8); ovarian serous and mucinous carcinoma (OC, P=97; M=5); pancreatic adenocarcinoma (PA, P=38; M=5) and gastric adenocarcinoma (GA, P=25; M=6).
Results: We identified IMP3 protein in the cytoplasm of tumor cells. A high percent of metastatic carcinomas compared to their primary tumors expressed IMP3 (P<0.05), including P: 21% (51/238) vs. M: 93% (26/28) of UC; P: 7% (5/70) vs. M: 83% (5/6) of EMU; P: 19% (4/21) vs. M: 80% (8/10) of PTC; P: 50% (5/10) vs. M: 80% (4/5) of SCC; P: 16% (25/159) vs. M: 78% (28/36) of RCC; P: 8% (2/24) vs. M: 48% (13/27) of DCB and P: 69% (54/78) vs. 91% (10/11) of SK. Other tumors including colonic, lung, prostatic, pancreatic, ovarian and gastric carcinomas showed no significant difference of IMP3 expression between primary and metastatic carcinomas. IMP3 expression was not found in any of benign tissue adjacent to the malignancies.
Conclusions: Our data provide important baseline information for IMP3 expression in different malignancies. As there is significant higher expression of IMP3 in metastatic cancers compared to their primary counterparts, IMP3 may have a potential prognostic value for thyroid, breast, uterus and skin cancers.

1543 Differential Tissue Expression of Androgen and Estrogen Related Proteins in Normal Weight and Obese Prostate Cancer Patients

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Background: Obesity is associated with an aggressive form of prostate cancer and changes in androgen and estrogen metabolism. Given the associations between obesity, circulating sex sterols and nuclear hormone receptor expression in prostate cancer, we hypothesized that changes in components of the sex steroid receptor axis may contribute to the clinical aggressiveness of prostate cancer in obese patients.
Design: A comprehensive database was assembled containing clinical and pathological variables (age, PSA, ethnicity, body mass index, Gleason score, stage, margin status, etc) from 541 patients (81 obese; 460 non-obese) treated with radical prostatectomy

at Cedars-Sinai Medical Center between 1994 and 2002. The final case set selected for study was comprised of 68 obese and 69 non-obese men. Tissue microarrays were constructed from representative case tissue blocks for study by standard immunhistochemical techniques using antibodies against androgen receptor (AR)(Dako clone AR441), PSA (Dako rabbit polyclonal), estrogen receptor α (ERα)(Ventana Confirm anti-estrogen, SP1), estrogen receptor β (ERβ)(Abcam clone PPG5/10), and aromatase (Santa Cruz Biotechnology CYP19). Semiquantitative analysis was performed, assessing benign and malignant epithelium and the stroma in proximity to each. Statistical analysis was performed.

Results: Body mass index correlated strongly with race, extra-capsular extension, and advanced pathologic stage. PSA, ERβ and aromatase were expressed in cancerous epithelial cells in most samples. Decreased expression of ERβ and aromatase in obese patients was observed in the stromal compartment surrounding non-cancerous acini.

Conclusions: We confirm the previously reported associations between obesity and aggressive clinical and pathologic features in our single-institution, urban teaching hospital. In comparing obese versus non-obese patients, there was no difference in expression of androgen or estrogen related proteins in cancerous epithelial cells. However, there was a down-regulation of ERα and aromatase in the stroma of obese patients. Our data suggests obesity may cause stromal changes in sex steroids which can affect prostate cancer growth via intracrine/paracrine mechanisms.

1544 The Angiogenesis Regulator & Notch Pathway Activator Delta like Ligand 4 (Dll4) Is Widely Expressed by Vascular & Neoplastic Cells in Human Cancer and Relocates to the Nucleus. An Immunohistochemical and In-Situ Hybridization Study

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Background: Dll4 induced by the Vascular Endothelial Growth Factor (VEGF) inactivates VEGF induced angiogenesis by negative feed-back. Dll4 block inhibits tumor growth in VEGF resistant mouse tumours. Furthermore, in addition to the antitumoral action of Dll4 alone, combined treatment with anti VEGF is more efficient. To address Dll4 protein expression, we generated D4/37 a monoclonal antibody (McAb) against the Dll4 intracellular domain (ICD).

Design: 22 breast & 24 bladder cancers, 20 meningiomas, 10 glioblastomas (GBs) & 9 tissue arrays containing colorectal, kidney, lung & oligodendroglial cancer and non-tumoral tissue were selcetedd for study. Immunostaining incuded antigen retrieval heating, pre incubation with D4/37, followed by En Vision (Dako, Denmark) detection. Ab7280 (Abcam, England) against Dll4 extracellular domain (ECD) was used as a control. In-situ hybridization (ISH) was performed on 22 breast and 24 bladder cancers.

Results: D4/37 McAb showed cytoplasmic and more occasional nuclear staining on GFP-Dll4-ICD but not Dll4-ECD-GFP transfectants. On the contrary, Dll4-ECD-GFP transfectants were immunostained by Ab7280 but not with D4/37. D4/37 and Ab7280 provided similar patterns of Dll4 expression. Intensity and number of positive cells were higher with D4/37. Dll4 cytoplasmic staining was frequent in vessels, neoplastic and inflammatory cells of most breast, bladder, kidney, colorectal & brain cancers. Nuclear expression was occasional. Non tumoral cells (epithelial, stromal and reactive astrocytes) showed Dll4 expression. Vessels highly expressed Dll4 mRNA. Neoplastic & inflammatory cells of breast, gallbladder cancers & non-neoplastic breast lobules displayed weak Dll4 mRNA expression. Our study has provided these new findings: 1) Dll4 protein & mRNA expression is not restricted to endothelial cells. Expression by neoplastic, inflammatory, epithelial and reactive cells suggest microenvironmental cells may play a role in angiogenesis. 2) Dll4 nuclear expression suggests Dll4 nuclear activity.

Conclusions: D4/37 allows the analysis of Dll4 protein expression in human tissues in clinical practice. Dll4 expression could allow patient stratification to take treatment decisions targeting Dll4 to block angiogenesis and stop tumoral growth.

1545 Does Epithelial-Mesenchymal Transition Underlie Breast Cancer Metastases?

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Background: Epithelial–mesenchymal transition (EMT) is a well-recognized developmental phenomenon during which epithelial cells lose many of their basic epithelial characteristics, such as cell to cell adherence and basal-apical polarity, and acquire properties that are typical of mesenchymal cells, such as motility. In EMT, vimentin (VIM) is the predominant intermediate filament expressed, while cytokeratin expression decreases. The role of EMT in cancer metastases is highly controversial. A direct comparison of morphologic and immunohistochemical features of EMT in primary breast carcinomas and their multifocal metastases has not been performed.

Design: We performed rapid autopsies (postmortem interval, 1-4 hours) on 15 consenting patients with metastatic breast carcinoma (BC). Single-patient tissue microarrays (TMAs) were constructed from the patients' archived primary BC and multiple different metastatic BCs harvested at autopsy. TMAs were assessed for evidence of sarcomatoid morphology, which would provide evidence for EMT. TMAs were labeled by immunohistochemistry (IHC) for estrogen receptor (ER), progesterone receptor (PR), HER-2, and CK5/6 to determine the primary BC's IHC surrogate profile corresponding to breast cancer subcategories defined by gene expression profiling. TMAs were labeled for vimentin (VIM) as an immunohistochemical marker of occult EMT, and VIM expression in primary BC (145 spots from 15 cases) and matched metastatic BC (778 spots from 180 different metastatic BCs) were compared.

Results: Eight primary BCs were Luminal A (ER+, HER-2-), five were Basal-like (ER-, PR-, HER-2-, CK5/6+) and two were HER-2 positive carcinomas. Review of 778 spots from 180 MBC revealed no evidence of sarcomatoid change. All 8 Luminal A cases were VIM negative in their primary BC and all metastatic BC. 6 cases were VIM positive, including 4 of 5 Basal-like cases and 2 of 2 HER-2 positive cases. In all 6 of these cases, VIM expression was detected in the primary BC as well as the metastatic BC. Upregulation of VIM expression was noted in only a subset of metastatic BC in 3 of these 6 cases. Although VIM labeling was variable between the different metastatic BCs, a metastatic site-specific preferential increase in VIM expression was not detected.

Conclusions: Morphologically and on immunolabeling for Vimentin, established metastatic BC show little evidence of EMT. If EMT plays a major role in breast cancer metastasis, it would have to be transient and reversible.

1546 Pulmonary Endotoxin Tolerance Protects Against Lung Injury While Maintaining Capacity To Clear Bacterial Pathogens

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Background: Repeated inhalation of high doses of endotoxin, such as that contained in grain dust has been shown to cause inflammation and airflow obstruction. Endotoxin inhalation both exacerbates and protects against allergic asthma, based on the timing and dose of exposure. Sublethal endotoxin exposure induces a state of refractoriness that protects against further endotoxin challenges, termed endotoxin tolerance (E-T). Little work has been done to characterize E-T in the lung. We sought to determine whether chronic endotoxin exposure can induce a state of E-T in the lung.

Design: To induce E-T, female BALB/c mice were exposed to 1μg of LPS (E.coli 011:B4) for four consecutive days by direct intratracheal installation. On day 5 mice received 10μg LPS intratracheally. Control, or non-tolerant mice received PBS on days 1-4 and 10μg LPS on day 5. Airways hyperresponsiveness (AHR) was measured 4 hours post final challenge. Mice were sacrificed and bronchoalveolar lavage fluid assayed for cytokines/chemokines.

Results: Chronic E-T resulted in a significant reduction in TNFα, however, IL-6 was significantly increased in the tolerant group at 2 hours. Comparable CXCL1 expression was measured in both groups, however CXCL2 was significantly decreased in the E-T group.

BAL fluid mediators at 2 hours post final challenge. * denotes p<0.05 compared to Non-tolerant group

	Non-tolerant (ng/mL ± SEM)	Tolerant (ng/mL ± SEM)
TNFα	7.7 ± 0.8	3.4 ± 0.3 (*)
IL-6	2.6 ± 0.4	5.6 ± 0.9 (*)
CXCL1	5.2 ± 0.6	4.9 ± 0.8
CXCL2	6.0 ± 1.2	2.3 ± 0.5 (*)
Tryptase	10.2 ± 2.2	2.4 ± 2.1 (*)
Cysteinyl leukotriene	0.6 ± 0.1	2.9 ± 1.1

No difference was seen in the number of neutrophils in the lung between E-T and non E-T mice. AHR was significantly attenuated in E-T mice (200% above PBS) compared to non E-T mice (650% above PBS). However, cysteinyl leukotriene,and tryptase in the BAL fluid were significantly increased in E-T mice.

Conclusions: Chronic endotoxin exposure in the lung induces a specific state of immunosuppression in the context of TNFα production and airways hyper-responsiveness. Other aspects of the immune response, such as IL-6 production and neutrophil recruitment remain intact. Our model also suggests that AHR is not mediated by cysteinyl leukotrienes or tryptase. Together, these data offer a possible mechanism of immune regulation that protects the lung from excessive inflammation (TNF), while retaining its ability to combat bacterial pathogens (neutrophil recruitment).

1547 Immunohistochemical Study of Hace1 in Wilms' Tumor and Breast Cancer Suggests a Pro-Tumorigenic Role for Its Nuclear Isoform

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Background: Hace1 is a cytoplasmic HECT E3 ubiquitin ligase we identified by cloning the 6q21 breakpoint of a translocation targeting the *Hace1* locus in Wilms' tumor (WT). Subsequent studies showed that *Hace1* is frequently inactivated epigenetically in WT, and decreased expression by RT-PCR was seen in multiple tumor types relative to patient-matched normal tissue. Also, *Hace1* null mice developed diverse spontaneous tumors. However, studies into the significance of Hace1 protein expression in primary tumors are needed.

Design: Using a mouse monoclonal antibody we developed, immunohistochemistry (IHC) was performed on ten WT cases along with matched normal kidney. We also performed Hace1 IHC on a tissue microarray (TMA) of 1378 interpretable breast cancer cases with associated prognostic data, including median follow-up time for non-relapsed patients of 9.5 years. Hace1 expression was scored separately for cytoplasmic and nuclear staining (0=negative; 1+=weak or <10% positive cells, 2+=strong >10% of cells, 3+=intense in >50% cells).

Results: Hace1 IHC of normal kidney showed strong 2+ cytoplasmic and nuclear staining of the tubular epithelium, while paired WT samples showed markedly less staining overall, with consistently negative cytoplasmic staining. However, in contrast to *in vitro* expression studies, 7/10 cases showed 2+ nuclear staining. Hace1 IHC of the breast cancer TMA showed widely variable levels of both cytoplasmic and nuclear Hace1 staining. Neither cytoplasmic nor nuclear Hace1 levels correlated with disease-free survival when analyzing all cases combined. However, when basal-like triple-negative cases (HER2-/ER-/PR-) were isolated, strong nuclear Hace1 (2-3+) showed a trend towards decreased disease-free survival (n=169, RR=1.68, p=0.086). Preliminary *in vitro* studies suggest that a truncated alternative transcript of Hace1 exists which localizes to the nucleus, the biological function of which is yet unknown.

Conclusions: An unexpected nuclear localization of Hace1 was observed in this IHC study of WT and breast cancer, and suggests a pro-tumorigenic role based on an

association with poor prognosis in basal-like breast cancer. Further studies using a larger cohort of the basal-like subtype is needed, and *in vitro* studies are needed to determine the biological role of nuclear Hace1.

1548 Detection of Chromosomal Anomalies in Chromophone Renal Cell Carcinoma Using Fluorescence In Situ Hybridization

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Background: Diagnostic separation of oncocytoma and chromophobe renal cell carcinoma is often difficult by light microscopy and immunohistochemistry. We investigated the ability of multi-target fluorescence in situ hybridization (FISH)- detected chromosomal anomalies to make this distinction.

Design: The study group consisted of 41 renal tumors, including 18 oncocytomas and 23 chromophobe renal cell carcinomas. FISH was performed on 5-micron paraffin-embedded tissue sections with centromeric probes to chromosomes 2, 6, and 10. Signals were counted in 100 nuclei from each tumor.

Results: One of 18 oncocytomas (6%) showed loss of chromosomes 6 and 10; no loss of chromosome 2 was detected. Conversely, the majority (70% (16/23)) of chromophobe renal cell carcinomas showed loss of at least one chromosome, and 35% (8/23) had loss of all three chromosomes. FISH abnormalities in chromophobe renal cell carcinomas included loss of chromosomes 2 (65%), 6 (61%), and 10 (39%).

Conclusions: Loss of chromosomes 2, 6, and 10 are frequent in chromophobe renal cell carcinomas, whereas oncocytoma ucommonly shows loss of chromosomes 6 and 10. FISH with centromere-specific probes to chromosomes 2, 6, and 10 appear to be useful adjuncts to other diagnostic.

1549 Holoclone and Non-Holoclone Derived Cell Lineage miRNA Analysis in Prostate Cancer

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Background: Prostate cancer is the second leading cause of cancer deaths in men. Stem-like cells have been identified in several malignancies including prostate cancer and are thought to drive primary tumorigenesis through self-renewal and differentiation. Additionally, persistence of stem cells post-therapeutic intervention has been proposed as an explanation for metastasis and recurrence. Holoclones are a tightly packed clone of small cells generally thought to contain stem cells and progenitors. MicroRNAs (miRNAs) are recently discovered small family of regulatory molecules that are associated with various malignancies. The aim of this study was to identify a profile of prostate cancer-associated miRNAs, and to derive holoclones from prostate cancer cell lines and to characterise the miRNA profile of these stem-like cells.

Design: In this study, meta-analysis was carried out to compare existing data on miRNA expression in prostate cancer and data was analysed using miRGen and miRNApath. The expression of a panel of known human miRNAs, was assessed in a group of prostate cell lines PWR-1E (normal), LNCaP (metastatic carcinoma), PC-3 (non-metastatic adenocarcinoma) using a quantitative Real Time TaqMan® PCR method.

Results: Meta-analysis identified common miRNA species in prostate cancer and the predicted gene targets and pathways were also assessed. Putative holoclones were generated from cell lines (LNCaP, PC-3) using a high salt-soft agar assay and LNCaP putative holoclones were kept alive for 24 days and PC-3 holoclones were kept alive for 6 days (LNCaP cell line represents a metastatic prostatic carcinoma and should contain a higher number of cancer stem cells). miRNA profiling was performed using 380 individual assays based on Stem Looped Primer PCR reactions.

Conclusions: Analysis of the data showed unique miRNA populations varied between each of the cells profiled. Future work will consist of further analysis on the expression of human miRNAs in prostate cancer cell lines and holoclone derived stem cells in order to identify prostate cancer stem cell-specific miRNA populations. Predicted gene targets and pathways will also be assessed in the cell lines and holoclones, and then compared to the meta-analysis study. Acknowlegements: Prostate Cancer Research Consortium CERVIVA (ICSRC)

1550 Comparison of Different Methods for Detecting K-ras Mutations in Colorectal Carcinomas

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Background: Recent data on colorectal carcinoma treatment using EGFR inhibitors have indicated that only patients with wild K-ras may respond. Thus, the analysis of K-ras status could evolve to a routine test. Most of the previous K-ras analyses have not included quantitative real time PCR (QRT-PCR) which is advocated as the preferred method in clinical labs. Indeed, there are very few studies that compare traditional methods, based on RFLP and sequencing, with QRT-PCR.

Design: We examined 120 samples, from 120 consecutive cases with resections of advanced stage colorectal adenocarcinomas (one sample per patient). DNA was extracted from paraffin sections, using the Qiamp DNA mini kit (Qiagen). Manual microdissection was performed, when required, to exclude overabundance of non-carcinomatous or necrotic tissues. Primers for PCR were designed according to the pertinent literature. Multiple PCR products were obtained from every sample. One was subjected to overnight RFLP analysis with BstNI and HaeIII restriction enzymes for mutations in codons 12 and 13, respectively. Digestion products were analysed on a 3% agarose gel electrophoresis. Another was followed by direct sequence analysis at both directions on an ABI 3730 sequencer. In addition, from a subset of 60 samples, PCR products were checked for k-ras mutations by QRT-PCR on a Corbett 6000 using a commercially available kit (DxS). This application was optimized in preliminary tests with the assistance of the kit's manufacturer.

Results: Mutant alleles were found in 42.3% of the samples. Most of the mutations (83%) were found at the codon 12 and fewer at the codon 13 (17%). The most frequent mutation was Gly12Asp (G>A) in 41% of mutated samples followed by the Gly12Val (G>T) in 36%. K-ras status as predicted by RFLP was confirmed by sequencing in all samples. Repeated sequencing was necessary in only few cases. QRT-PCR gave overall similar results. However, in 4 cases it did not detect mutations found by RFLP and sequencing.

Conclusions: QRT-PCR using a commercially available kit is an accurate method for K-ras analysis in routine material. It can generate results faster, but it is not superior to RFLP and sequencing, which could be regarded as valid, less expensive alternatives and as the "gold standards" since there were cases with mutant k-ras not detectable by QRT-PCR.

1551 Stem Cell Protein Piwil2 Variant PL2V60 Regulates Precancerous Stem Cell Proliferation

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Background: During the tumor development, a germline stem cell protein piwil2 appears to play an important role, because it is silenced normally in adult tissues except for in the GS cells but activated ectopically in various types of tumor. Recently, we have reported that piwil2 is stably expressed in pCSCs derived from lymphoma. Knockdown of piwil2 mRNA using piwil2-specific siRNA reduced pCSC expansion *in vitro*. However, transduction of *piwil2* gene into stem/progenitor cells induced proliferation-associated cell death (PACD), which is strikingly contradictory to its role in pCSCs. Therefore, we hypothesized that the proliferation of pCSCs might be regulated by piwil2 variants rather than whole piwil2.

Design: First, we determined whether the transcripts of piwil2 or its variants were expressed in pCSCs, using piwil2-specific Gene Exon Array (GEA) RT-PCR. Then, the piwil2 variants expressed in pCSCs were quantitatively defined by Western-blot, using a novel antibody to the piwil2 peptide common for its variants, which is generated by us. Finally, the function of piwil2 variants expressed in pCSCs was verified using RNAi and biochemistry approaches.

Results: GEA RT-PCR analysis of 3 clones of pCSCs reveal that whole piwil2 did not express in pCSCs but its potential variants, which appeared to be truncated at 5'-end of transcripts. The peptide-affinity purified polyclonal antibody to a common peptide of piwil2 variants could react with piwil2 and several variants, designated as PL2V80, 65, 60, 52, and 42, which were expressed in both murine testis and human tumor cell lines. Western-blot analysis revealed that only PL2V60 was strongly expressed in pCSCs (3 ~ 6 units; normalized against β-actin of the same samples), and piwil2 and other variants were not significantly detectable except for PLV50, which was detected in trace amount in pCSCs. As control, the PL2V60 was almost not detectable in normal splenocytes (~0.05 units). Knockdown of PL2V60 mRNA and protein significantly inhibited pCSC proliferation *in vitro*. Moreover, PL2V60 expression was not limited to hematopoietic tumor cells; it was broadly detected in various types of human tumors such as breast and cervical cancer in a high level.

Conclusions: Our study indicates that PL2V60 is ectopically expressed in pCSCs, regulating their proliferation, and that it has the potential to be used as a novel biomarker for early detection and intervention of cancer.

1552 Lewis B (LeB) Antigen Expression Is Lower in Breast Carcinoma and Is Associated with Hormone Receptor Status and Histological Type

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Background: Characterization of cell surface expression of antigens such as LeB, is regarded a prerequisite for successful antibody-based cancer immunotherapy. Monoclonal antibody (mAb) 58-1066 was raised against LeB. To assess LeB expression profile in breast carcinoma and normal samples, further assessing it as a potential target for immunotherapy.

Design: Paraffin blocks from 106 high grade breast carcinoma specimens from the files of D. Pathology, Hospital das Clinicas, University of Sao Paulo, Brazil and 86 consecutive cases of carcinoma and non-neoplastic breast tissue from D. Pathology, Hospital de Clinicas Luzia de Pinho Melo, UNIFESP, Brazil. Immunohistochemistry was performed in Tissue Microarray samples: primary antibody: mAb58-1066; amplification system: Novolink, short polymer-base peroxidase method; semi-quantitation: estimation of percentage of membranous positive cells, classifying according to 10% increments as well as to intensity.

Results: Membranous immunostaining was seen in 80.6% and 22.3% of non-neoplastic and neoplastic samples, respectively. Non-neoplastic samples showed a higher amount of immunopositivite cells than carcinomas (means: 31.6% vs 4.5%, P<0.001). Intensity of membranous staining was higher in non-neoplastic samples ((0): 16.1% , (1): 29% , (2): 54.9%) versus carcinomas ((0): 77.7% , (1): 16.3% , (2): 6%), p<0.001. LeB expression was higher in PR+ versus PR- breast carcinomas (24.8% vs 11.9%, p=0.001) and was more common in lobular versus ductal type (24.3% vs 19.3%, p=0.047). There was a trend for association of ER+ and higher LeB expression (22.3% vs 13%, p=0.077). HER-2 status (p=0,419), locorregional spread (pT, p=0,506 and pN, p=0,366) and histological grade (p=0,685) were not found related to LeB immuno-expression.

Conclusions: LeB membranous expression was seen in a subset of breast carcinomas, preferentially lobular and progesterone receptor positive lesions, with a borderline association with estrogen receptor status. Further studies should assess a possible therapeutic role of mAb 58-1066 in these subtypes of breast carcinomas. This study is supported by grants from ReceptaBiopharma and FINEP, Brazil.

1553 *c-Jun* Amplification and Overexpression Are Oncogenic in Liposarcoma but Not Sufficient To Inhibit Adipocytic Differentiation

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Background: The c-Jun proto-oncogene is amplified and overexpressed in dedifferentiated liposarcoma (DDLPS). c-Jun overexpression has been hypothesized to mediate the transition from well-differentiated liposarcoma (WDLPS) to DDLPS. However, we previously found that c-Jun is frequently expressed in the well-differentiated portions of DDLPS. We sought to further evaluate the role of c-Jun in liposarcoma by mapping the genomic location of the c-Jun amplicon in primary DDLPS and by performing functional studies with a cell line derived from a DDLPS with c-Jun amplification.

Design: We performed FISH for MDM2 and c-Jun on metaphase spreads from DDLPS samples with known c-Jun amplification. We also performed FISH for c-Jun on well-differentiated components of DDLPS with known c-Jun amplification. We derived a cell line (LP6) from a DDLPS with c-Jun amplification and infected the cells with lentivirus encoding shRNA to c-Jun or GFP as a negative control. We tested the effect of c-Jun knockdown on the ability of LP6 cells to grow in tissue culture and to form tumors in nude mice.

Results: We have identified a case of DDLPS in which the well-differentiated component exhibits high-level c-Jun amplification, suggesting that c-Jun amplification does not block adipocytic differentiation. When c-Jun is amplified in DDLPS, it is frequently interspersed with amplified MDM2 on ring and giant marker chromosomes, indicating that the two genes were amplified at the same time. c-Jun knockdown by two different shRNAs in LP6 cells reduces viable cell number in vitro. In nude mice, LP6 cells with stable c-Jun knockdown form tumors that are significantly smaller than control LP6 cells after subcutaneous injection. Tumors from c-Jun knockdown cells exhibit a lower Ki67 rate than controls, but c-Jun knockdown in LP6 cells does not result in the extensive lipid accumulation characteristic of WDLPS.

Conclusions: We propose that c-Jun amplification is oncogenic in liposarcomas, but frequently occurs at the same time as MDM2 amplification and does not directly inhibit adipocytic differentiation of the liposarcoma cells.

1554 The ALT Phenotype in Breast Carcinoma Is Associated with HER-2 Overexpression

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Background: The majority of human carcinomas possess unlimited replicative capacity in part due to telomerase, an enzyme that maintains chromosomal telomere length. However, approximately 10-15% of human cancers do not show evidence of telomerase activity, and maintain telomere lengths by a recombination-based mechanism termed alternative lengthening of telomeres (ALT). The ALT pathway leads to marked heterogeneity in telomere lengths within individual cells, and is also distinguished by the presence of so-called ALT-associated promyelocytic leukemia (PML) protein nuclear bodies (APB) that contain large amounts of extra-chromosomal telomeric DNA, PML protein and other proteins involved in telomere-binding, DNA replication, and recombination. The ALT phenotype is commonly identified in adult sarcomas and testicular germ cell tumors, but is very rare in carcinomas. The frequency of the ALT phenotype in molecular subclasses of breast carcinoma has not been systematically evaluated.

Design: Tissue microarrays (TMAs) were created from 71 invasive ductal carcinomas (IDCs) of the breast which had been previously characterized for ER, PR, HER-2, EGFR, and CK5/6 expression. The cases included four distinct groups of IDCs having surrogate IHC profiles corresponding to categories defined by gene expression profiling {17 Luminal A (ER+, HER-2-), 7 Luminal B (ER and/or PR+, HER-2+), 14 HER-2+ (ER-, PR-, HER-2+), 21 basal-like carcinomas (BLC) (ER-, PR-, HER-2-, CK5/6 and/or EGFR+), and 12 unclassifiable triple negative carcinomas (TNC) (ER-, PR-, HER-2-, CK5/6-, EGFR-)}. Using a previously described fluorescence *in situ* method (Am J Pathol 2002;160:1259-66), telomere lengths in the IDCs were assessed.

Results: The ALT phenotype was identified in 3 of 21 HER-2 positive cases (Luminal B and HER-2+), but in none of the 17 Luminal A cases, 21 BLC cases, or 12 TNC cases (p=0.023).

Conclusions: In IDC, the ALT phenotype occurs preferentially in a subset of cancers with HER-2 overexpression. This association suggests that the ALT phenotype, which reflects DNA sequence amplification via recombination, may be correlated with HER-2 amplification through a common underlying mechanism. Since cancers utilizing the ALT pathway are predicted to be resistant to therapies based on telomerase inhibition, these results may have therapeutic consequences. As the presence of the ALT phenotype has prognostic significance in some cancers (e.g. Cancer Res 2006;66:8918-24), its prognostic implications in IDC of the breast should be further studied.

1555 CD87, a Prognostic and Diagnostic Flow Cytometry Marker in Myelodysplastic Syndrome

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Background: We have previously demonstrated the maturing myeloid population in bone marrow shows loss of CD87 (urokinase plasminogen activator receptor) expression in myelodysplastic syndrome (MDS). In the current study, we assessed the potential role of CD87 as a prognostic tool.

Design: We performed a retrospective chart review of 28 patients who had a flow cytometry panel performed for the diagnosis of MDS between 2006 and 2008. The panel assessed myeloid cells for surface expression of CD13, CD16, CD10, CD87, HLADR and CD34. All patients had at least one of the following: positive flow cytometry for MDS, positive cytogenetics, and/or evidence of MDS on bone marrow biopsy. A retrospective chart review was performed and the hematocrit (HCT), white blood cell count (WBC) and platelet count (PLT) at patient presentation were obtained. We correlated the expression of CD10, in addition to CD87, with the HCT, WBC and PLT counts given that decreased CD10 expression is currently a more widely accepted marker for MDS.

Results: Of the 28 patients (14 male vs. 14 female, mean age 65, range 27-81), 18 had a decreased expression of CD87 (≤30). This group of patients had a significantly lower HCT and WBC when compared to the 10 patients with normal expression of CD87. The patients with a decreased CD87 had a average HCT of 25.2%± 6.3 versus 31.5%± 6.8 in the patients with normal CD87 expression (p=0.02). The decreased CD87 group also had a lower WBC the group with normal CD87 (3.3 K/uL ± 1.4vs. 6.2 K/uL ± 4.4, p=0.008). There was no significant difference in the platelet values (163 K/uL vs. 118 K/uL, p=0.35) between patients with decreased and normal CD87. The comparison of the 24 patients with decreased CD10 expression (≤30) and the four patients with normal CD10 expression did not show any significant differences in the HCT, WBC or PLT counts.

Conclusions: Loss of CD87 expression is not only useful for the diagnosis of MDS using flow cytometry, it is also associated with more severe anemia and leukopenia at clinical presentation. Expression of CD10, a more widely used flow cytometric marker in the diagnosis of MDS, showed no correlation with HCT, WBC and PLT counts. Additional prospective studies of CD87 and other flow cytometry markers are necessary to further elucidate the prognostic implications of loss of CD87, as well as other aberrantly expressed surface markers in MDS.

1556 Expression of Abelson-Interactor Protein 1 (ABI-1) in Breast Cancer and Its Association with Phospho-AKT

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Background: Breast cancer is the second leading cause of cancer-related mortality. Most deaths are due to metastasis. Accumulating data suggest that actin and cytoskeleton-associated proteins might be key players in tumor metastasis. Abelson-interactor protein 1 (ABI-1) is an adaptor protein involved in actin polymerization and cell migration, and it is widely expressed in human and mouse tissue. Previous *in vitro* work in our lab suggested that ABI-1 is a positive regulator of breast cancer proliferation, migration and invasion. In the present study, we explore the expression and cellular distribution pattern of ABI-1 in breast cancers using tissue microarrays (TMAs) and further identify its association with phospho-AKT (P-AKT).

Design: Commercially available breast cancer TMAs were obtained. In total, 202 cases of invasive breast cancers in duplicate cores were available to study from four different TMA sets. All cases were provided with clinicopathological prognostic parameters. Antibodies to human ABI-1, phospho-AKT and Ki67 were used for the study. Immunohistochemical staining was performed using a routine protocol. The intensity of staining of ABI-1 and P-AKT and the percentage of nuclei staining with Ki67 was evaluated by two independent pathologists.

Results: ABI-1 was detected in the cytoplasm of breast cancer cells. Out of 202 cases, 98 (49%) showed high expression of ABI-1 and 104 (51%) showed low expression. ABI-1 expression is closely related to the expression of P-AKT (*P*-value 0.011). There was no correlation between ABI-1 expression and other clinicopathologic parameters, including tumor grade, Ki67, ER/PR status, Her-2/Neu and stage. Data mining using publicly available breast cancer gene expression array databases showed that ABI-1 is negatively correlated to ER/PR status and survival, and positively correlated to tumor grade.

Conclusions: To our knowledge, this is the first study evaluating ABI-1 expression in breast cancer using TMAs. ABI-1 was localized to the cytoplasm of breast cancer cells and showed different expression levels. ABI-1 expression is correlated to P-AKT in our data set, which is a predictor of poor prognosis in most cancers. Combined with the gene expression array data, we believe that ABI-1 may be a candidate gene for predicting poor prognosis in breast cancer and is involved in spread of breast cancer cells.

1557 A New Phosphatase Involved in Human Carcinomas: Laforin Is Downregulated in a Large Set of Tumors, Supporting Its Role as Tumor Suppressor

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Background: A large number of molecular alterations have been described in cancer. Importantly, very few phosphatases, like PTEN, have been described so far to be involved in human tumors. Recently, Laforin was associated with T-Lymphomas in an inmunocompromised mouse model. Laforin is a ubiquitous protein encoded by the EPM2A gene with a critical role in glycogen metabolism, and is mutated in at least 50% of the patients with progressive myoclonus epilepsy (Lafora disease). This protein contains a dual-specificity protein phosphatase domain, and has been linked to GSK3 β dephosphorylation.

Design: We have studied the expression levels of Laforin by WB and real-time PCR in 58 Colorectal Adenocarcinomas, 15 Clear-Cell Renal Carcinomas, 10 Astrocytomas, 15 Lung carcinomas and 25 Breast Carcinomas. Colon, kidney and lung samples were compared with normal adjacent tissue, Breast carcinomas and Astrocytomas were compared to pooled normal tissue. Five adenomas adjacent to colorectal adenocarcinomas were also studied. Cell lines derived from breast (MDA-MB-231 and 435) and colon cancer (HCA7 and HT29) were infected with retroviruses to induce overexpression of Laforin, and cell growth was measured by the Population Doubling technique.

Results: Laforin protein was reduced or absent in 80% of colorectal adenocarcinomas, 80% clear-cell renal carcinomas, and 40% breast carcinomas. Similar results were

obtained by real-time PCR. No concomitant increase in GSK3 β phosphorylation was observed. Conversely, no significant decrease in Laforin expression was observed in Astrocytomas. Laforin mRNA and protein levels were lower in adenocarcinoma than in polyps, and lower in polyps than in normal tissue, hinting a gradual reduction in Laforin during the development of colorectal cancer. Finally, the overexpression of Laforin in transformed cell lines reduced the proliferation index.
Conclusions: A striking lack of Laforin protein was observed in a large number of colon and kidney carcinomas, thus presenting a role for a novel phosphatase in a large set of human tumors. We propose that Laforin may be a tumor suppressor gene that is downregulated in early steps of tumor development.

1558 Altered Expression Pattern of Myocyte Enhancer Factor (MEF2) in Benign and Malignant Cartilaginous Tumors

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Background: MEF2 consists of four isoforms-MEF2A, 2B, 2C and 2D which are important regulators for tissue differentiation and signal responsiveness, in skeletal / cardiac muscle, endothelial cells, brain and cartilage. Suppression of MEF2C resulted in impairment of cartilage development in MEF2C +/- mice and this effect can be augmented by deletion of one MEF2D allele in MEF2C+/- mice. MEF2C over-expression caused precocious chondrocyte hypertrophy and dwarfism. We studied MEF2 expression in benign and malignant cartilaginous lesions with a desire to explore differential expression and thereby evaluate its role in the development of these lesions.
Design: Enchondromas (7), chondromatosis (3), grade I (12), grade II (8) and grade III/ dedifferentiated (9) chondrosarcomas were selected for analysis. MEF2 antibody (C21, Santa Cruz, CA) was tested with appropriate controls. Staining results were assessed for intensity (strong, intermediate, weak or negative) and extent. Also noted was staining localization (cytoplasmic and/or nuclear staining).
Results: In benign chondroma and low grade chondrosarcoma , the staining of MEF2 is weaker and nuclear in cellular area but negative in hypocellular hyaline cartilaginous areas. In Grade II chondrosarcoma and chondromatosis the nuclear staining was diffuse and strong. In dedifferentiated chondrosarcoma , MEF2 was expressed not only in the malignant chondrocytes but also in the spindle sarcomatous areas where staining became preferentially cytoplasmic. In all lesions, the staining was prominent in cellular areas but weak or negative in hypocellular hyaline cartilaginous areas.
Conclusions: MEF2 was strongly expressed in cellular proliferating chondrocytes compared to the mature appearing cartilaginous areas. MEF2 expression correlated with cellularity of a lesion whether benign or malignant. In dedifferentiated chondrosarcoma preferential cytoplasmic staining was noted. Our results suggest MEF2 is involved in proliferation of chondrocytes and in the oncogenesis of cartilaginous tumors in humans.

1559 Regulation of VEGF-Induced Vascular Permeability and VEGFR2 Signaling by Thrombospondin-1

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Background: VEGF is a well-established stimulator of vascular permeability and angiogenesis. VEGF-induced vascular hyperpermeability is an important characteristic of many disease states. Thrombospondin-1 (TSP-1) is a potent angiogenic inhibitor. Therapeutics based on the antiangiogenic domain of TSP-1, designated the three TSP-1 type 1 repeats (3TSR), have shown promising antiangiogenic and anti-tumor efficacy. We performed this study to characterize the regulatory effects of TSP-1 on VEGF-induced vascular permeability and VEGF receptor 2 (VEGFR2) signaling.
Design: Basal-level and VEGF-induced permeability were evaluated in TSP-1-null mice and 3TSR-treated FVB mice using the Miles assay. Lung extracts from TSP-1-null mice and 3TSR-treated mice with or without VEGF iv injection were analyzed for VEGFR2 phosphorylation at tyrosine 1173. Wild-type and/or buffer-treated mice were used as control in the above experiments. VEGFR2 phosphorylation, including time course and dose response, was further characterized in vitro using 3TSR-treated human dermal microvascular endothelial cells (HDMEC) and vascular endothelial cells isolated from TSP-1-null mice.
Results: Systemic treatment of wild-type mice with 3TSR significantly decreased VEGF-induced permeability ($p<0.01$). VEGF-stimulated VEGFR2 phosphorylation was also significantly decreased in lung extracts from 3TSR-treated mice ($p=0.03$). Moreover, 3TSR significantly decreased VEGF-stimulated VEGFR2 phosphorylation in HDMECs in culture. We further performed the Miles assay in wild-type and TSP-1-null mice. Basal levels of permeability in multiple organs were equivalent in the two strains. Surprisingly, VEGF-induced permeability was significantly decreased in TSP-1-null mice ($p<0.03$) as compared to the wild-type control mice. In addition, systemic treatment of TSP-1-null and wild-type mice with VEGF produced lower levels of VEGFR2 phosphorylation in lung extracts of TSP-1-null mice. Vascular endothelial cells from TSP-1-null mice also showed significantly decreased VEGFR2 phosphorylation upon VEGF treatment. Whereas the magnitude of the response to VEGF is reduced in TSP-1-null endothelial cells as compared to their wild-type counterparts, the time course and dose response were comparable.
Conclusions: VEGF-induced vascular permeability and VEGFR2 phosphorylation display a biphasic response to TSP-1 concentration in tissues and isolated endothelial cells.

1560 Adenosine but Not Diazoxide or Ischemic Preconditioning Significantly Rescues Myocardial Injury in HIF-1alpha Deficient Heart

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Background: Ischemic preconditioning (IPC) has a profound protective effect against a subsequent prolonged episode of ischemia–reperfusion. Many mechanisms have been proposed in the process, including mitochondrial KATP channel or reactive oxygen species (ROS) dependent or independent. As a master transcription factor in responsive to hypoxia, HIF-1 is highly possible to be involved in preconditioning protection against ischemia-reperfusion.
Design: The study is based on Langendorff perfusion system performed with hearts from HIF-1alpha wild-type (WT) mice and mice heterozygous for a null allele at the locus encoding HIF-1alpha (HET). The hearts were subjected to IPC (two cycles of 5 min ischaemia/5 min reperfusion), followed by 30 min ischemia (I30) and reperfusion (45 or 120 minutes). ROS production in isolated cardiac mitochondria was measured by a chemiluminescence assay. Apoptosis and infarct size were assessed by TUNEL assay, cleaved caspase 3 immunohistochemistry, and triphenyltetrazolium chloride staining.
Results: In our previous study, IPC was shown to increase mitochondrial production of hydrogen peroxide and superoxide in WT but not in HET hearts. In current study, the IPC was associated with 14% reduction of hydrogen peroxide and 29% reduction of superoxide in WT hearts with prolonged ischemia followed early (5 minutes) reperfusion (WTIPC+I30/R5 vs. WTI30/R5), whereas in HET hearts it did not reduce but increased mitochondrial production of hydrogen peroxide and superoxide in 140% and 5% respectively (HETIPC+I30/R5 vs. HETI30/R5). In addition, preconditioning by IPC or diazoxide limited myocardial apoptosis and infarct size after prolonged ischemia–reperfusion (I30/R45 or R120) in WT hearts, but not in HET hearts. In contrast, Adenosine preconditioning reduced apoptosis and infarct size after prolonged ischemia–reperfusion in both WT and HET hearts.
Conclusions: Ischemia preconditioning can prevent myocardial injury in prolonged ischemic events, which likely involves small amount of ROS production to lessen subsequent ROS bursting. However, such a mechanism is disrupted in HIF-1alpha deficient heart. Adenosine but not diazoxide or ischemic preconditioning can significantly rescue myocardial injury in HIF-1alpha deficient heart with prolong ischemic attack.

1561 Loss of Heterozygosities in Barrett Esophagus, Dysplasia and Adenocarcinoma

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Background: Gastroesophageal biopsy (GEB) is widely utilized to evaluate gastroesophageal lesions that help guide surveillance for Barrett esophagus (BE) and low grade dysplasia (LGD) and treatment for high grade dysplasia (HGD) and adenocarcinoma. We evaluated whether there is a progressive accumulation of genomic mutations from BE to malignant transformation and studied which genomic mutations are important in the transformation with GEB specimens.
Design: Representative cells of 34 GEBs including normal mucosa, BE, LGD, HGD and adenocarcinoma were microdissected and DNA from these cells were extracted. LOH was quantitatively determined for a broad panel of 17 microsatellite repeat markers targeting 10 tumor suppressor genes by PCR followed by automated capillary electrophoresis.
Results:

Table 1. Progressive accumulation of LOHs in gastroesophageal lesions

Group	LOHs (Mean ± SD)	LOHs Range	P Value
Normal Mucosa	0.00 ± 0.00	0	
BE	0.45 ± 0.69	0 - 2	0.053
LGD	2.40 ± 0.89	1 - 3	0.005**
HGD	4.75 ± 1.50	4 - 7	0.043*
Adenocarcinoma	5.25 ± 1.50	4 - 7	0.654

Student's T test. *: $P < 0.05$; **: $P < 0.01$.

Table 2. Frequency (%) of LOHs in gastroesophageal lesions

Chromosome	Normal Mucosa	BE	LGD	HGD	Adenocarcinoma
1p36	0	20*	40	75	50
3p24	0	9*	20	50	25
5q23	0	0	0	25*	75*
9p21	0	9*	20	50	67
10q23	0	0	40**	25	25
17p13	0	9*	40*	50	50
17q12	0	0	40**	50	25
17q21	0	0	0	67*	67
21q22	0	0	0	0	67**
22q13	0	0	0	0	0

Yates's correction test. *: $P < 0.05$; **: $P < 0.01$.

Conclusions: 1. There is a progressive accumulation of LOHs from BE to adenocarcinoma. 2. LOHs at 1p36 (CMM1 and MYCL1), 3p24 (VHL and PPARγ), 9p21 (p16 and p14arf), and 17p13 (p53) may play an important role in BE, LOHs at 10q23 (pTEN), 17p13 and 17q12 (HER2/neu and NF1) in LGD, LOHs at 5q23 (APC and MCC) and 17q21 (NME1 and BRCA1) in HGD, and LOHs at 5q23 and 21q22 (TFF1 and PSEN2) in adenocarcinoma transformation. 3. Detection of LOHs targeting tumor suppressor genes can be useful in evaluating gastroesophageal lesions, studying oncogenesis of gastroesophageal adenocarcinoma, and determining surveillance for BE and LGD and/or treatment for HGD and adenocarcinoma.