express progenitor cell markers. Studies are undergoing to identify the cell of origin of the retinoblastoma stem cells. Our results suggest that retinoblastoma stem cells are possible effective therapeutic targets.

1482 Immune Response of Retinoblastoma after Gene Transfer Using Adnoviral-Mediated Delivery of Thymidine Kinase Followed by Ganciclovir

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Background: The purpose of this study was to evaluate potential local and systemic immune responses to the adenoviral vector used for treatment of patients with retinoblastoma vitreous tumor seeding.

Design: An IBC, IRB, RAC and FDA approved pilot study of intra-patient dose escalation was initiated to examine the intravitreal injections of an adenoviral vector containing a herpes thymidine kinase gene (AdV-TK) followed by systemic administration of ganciclovir to treat retinoblastoma. Blood samples were drawn weekly before and after the intraocular injections and the T cells were examined for evidence of adenovirus-specific reactivity. All patients eventually required enucleation and specimens were compared and immunohistochemically to retinoblastoma enucleated eyes without any previous treatment to identify the cellular inflammatory component and antigen presenting cells in these eyes using the following antibodies: CD3, CD5, CD43, TdT, CD68, L-26, CD138, CD21, CD23 and CD1a.

Results: No patient had an increase in antibody titer to adenovirus following therapy. Blood samples of 4 patients had no changes in the precursor frequency of adenovirus–specific T cells. Control (after conjunctival adenoviral infection) blood sample showed marked changes in frequency of adenovirus-specific T cells. The IHC results show that there was statistical significant difference between the ocular response in gene transferred and non-treated retinoblastoma eyes. Gene tranduced tumors showed statistically increased amount of T-cells labeling with CD3, CD43, and TdT; of B-cells labeling with CD20 and plasma cells labeling with CD138 when compared with non treated retinoblastoma eyes. The tumors show no significant differences between the two groups.

Conclusions: T-cell response and B-cells with plasma cells in intraocular structures in gene therapy treated eyes is prominent compared to the control eyes. Plasma cells were present in the intraocular structures and not in tumors predominantly in gene tx patients. The results suggest that the type and location of inflammatory cells may not only play a role in therapy related toxicity but also in anti-tumor response.

1483 Variation of Monosomy 3 within Uveal Melanoma

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Background: Uveal malignant melanoma is the most common primary intraocular malignancy in the adult population. Determining the most significant prognostic variables has been elusive but important in order to stratify patients for metastasis surveillance and possible initiation of chemotherapy or immunotherapy. Chromosomal changes of prognostic significance have been found and include monosomy 3 and alterations of 8q and 6p. While traditionally, this tumor (unlike almost all other tumors) is treated without a histopathologic diagnosis, some investigators have recently begun to acquire tissue using FNA or vitrector needles, some using a transvitreal and others a transscleral approach. Previous investigators have shown that there can be variability within a given tumor for the presence of monosomy 3. Our hypothesis was that there may be differing results for monosomy 3 detection depending on whether apex or base tumoral tissue is obtained, and this difference might impact on the proper stratification of patients for non-operative treatment.

Design: Paraffin embedded enucleated eyes from 19 patients with primary uveal melanoma were studied by FISH for monosomy 3, both in the tumoral apex and base. FISH was performed using directly labeled pericentromeric chromosome enumeration probes (CEP3 and CEP4) labeled with SpectrumOrange and SpectrumGreen respectively. Twenty nuclei in 2 representative fields in the preselected apex and base areas were counted; only nuclei with at least 2 reference CEP4 signals were counted. Monosomy 3 was determined to be present if the CEP3/CEP4 ratio was ≤ 0.7.

Results: 16 of the 19 samples (84%) revealed concordance of monosomy 3 (7 of 16, 44%) or of disomy 3 (9 of 16, 56%). In 3 samples (16%), there was discordance of monosomy 3 status between the tumor base (monosomy 3) and tumor apex (disomy 3). Lack of concordance between the base and apex did not correlate with morphologic cell type. All three discordant cases demonstrated the monosomy 3 at the base with disomy at the apex.

Conclusions: Uveal melanoma displays tumor heterogeneity, genetically as well as morphologically, as evidenced by variation of monosomy 3 status within uveal melanoma. Fine needle aspiration biopsy samples obtained from the tumor base (scleral approach) vs. apex (transvitreal approach) may yield disparate results, which could effect therapeutic decisions.

Pathobiology

1484 Dysplastic Tubules and Mitochondrial DNA Mutations Support a Precursor Lesion to Renal Cell Carcinoma

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Background: Renal cell carcinoma (RCC) is the most common malignancy of the kidney and accounts for 3% of all malignancies in adults. Unlike many other organ systems, a precursor lesion for an invasive carcinoma has not been described in the kidney. In our experience, we have occasionally observed the presence of dysplastic renal tubules (DT) adjacent or remote to RCC. These tubules display a range of features of histologic atypia, including clear cytoplasm, prominent nucleoli, irregular nuclear membranes and variation in nuclear size without architectural abnormalities. Mitochondrial (mt) DNA mutations have been described in RCC. However, the molecular mutation profile of DT has not been reported. In this study, we investigated the mtDNA mutation profile of DT and compared the findings with normal renal tissue and RCC.

Design: A total of 35 cases of RCC were retrieved and reviewed. Eight of the 35 cases (23%) had DT based on histomorphologic features. The control groups include 5 normal renal tubules (NRT) in autopsy kidneys and 8 RCC. Following review of the cases, representative areas of RCC and DT were selected from each case. Three areas from the RCC and DT on each case were microdissected and DNA was extracted. We analyzed mutations of the mtDNA regulatory region or D-loop at 3 sites (D310, D512, and D4977) using PCR/electrophoresis.

Results: All cases of RCC (100%) showed mutations of mtDNA, the most common being mutation of D4977 (8/8 cases). All DT (100%) had mutations of mtDNA, with the most common being D4977 (7/8 cases). NRT showed mutation in 2 cases, but at a much lower rate than DT.

Number of cases positive for mtDNA mutation and rate of mutation

	D310		D512		D4977	
	Number of cases positive	Average rate of mutations	Number of cases positive	Average rate of mutations	Number of cases positive	Average rate of mutations
RCC	7	2	5	0.63	8	1.63
DT	6	1.1	2	0.33	7	1
NRT	2	0.63	0	0	2	0.63

RCC (renal cell carcinoma), DT (dysplastic tubules), NRT (normal renal tubules)

Conclusions: We demonstrated that mtDNA mutations are present in DT and in RCC, but not in NRT. These findings are supportive that DT may be a precursor of invasive renal cell carcinoma. Further investigation of oncogene expression in DT may also be helpful to better define the concept of "dysplastic" renal tubules as a precursor lesion in RCC.

1485 Carcinosarcomas Exhibit EMT – A Phenomenon of Tumor Progression Unrelated to Metastasis

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Background: The importance of epithelial mesenchymal transition (EMT) in tumor progression is controversial although its occurrence has been well documented in many model systems. Questions concerning its transient nature, its reversibility and most importantly its role in metastasis have been debated. Human carcinosarcomas of diverse types exhibit EMT in which there is a progression from a carcinomatous gene expression profile to a sarcomatous one and a concomitant pathological change from epithelial to mesenchymal histology.

Design: We decided to study EMT from both an observational as well as experimental perspective to address its role in metastasis. We studied 20 cases of human carcinosarcomas by E-cadherin, cytokeratin, β-catenin and vimentin IHC. We also used a transgenic murine model of breast cancer (WAP-*myc*) which exhibits EMT and pulmonary metastasis. We transfected cells from this model *ex vivo* with WAP-*cre* and lox (β-gal) reporter and with FSP-*cre* and lox reporter constructs and reinjected the cells into the cleared mammary fat pads of wild type mice. WAP is a promoter active in mammary epithelial cells whereas FSP is a promoter active in mesenchymal cells. Once the promoter becomes active in the constructs it causes an irreversible expression of the reporter.

Results: Each of the human carcinosarcomas exhibited IHC evidence of biphasic immunoreactivity yet their corresponding metastases were carcinomatous. The WAP-*myc*/WAP-*cre*/lox carcinoma cells exhibited primary tumors where both the carcinomatous as well as the sarcomatous areas expressed β-gal. The corresponding pulmonary metastases also expressed β-gal and were carcinomatous. The WAP-*myc*/FSP-*cre*/lox carcinoma cells exhibited identical primary tumors as the previous WAP-*myc*/WAP-*cre*/lox except that only the sarcomatous regions expressed β-gal. Their pulmonary metastases were exclusively carcinomatous and non-expressive of β-gal.

Conclusions: In both observational and experimental studies, there is clear evidence that EMT occurs. However the experimental studies shed further light upon the nature of EMT. EMT appears to be unidirectional (carcinoma to sarcoma and not vice versa), not derived from a stem cell switch, not transient and not metastasis promoting because if any of these latter features were true, the WAP-*myc*/WAP-*cre*/lox and the WAP-*myc*/FSP-*cre*/lox mammary carcinomas would not exhibit their pattern of β-gal expression in their primary tumors and metastases.

1486 Predilection of Pancreatic Ductal Adenocarcinoma Cells To Form Duct-Like Structures in Vascular and Perineural Spaces, Mimicking Normal Ducts and PanIN: A Peculiar Form of Tumor-Stroma Interaction

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Background: The tremendous ability of pancreatic ductal adenocarcinoma (DA) cells to rapidly disseminate, despite its relatively well differentiated morphology, is one of the most intriguing questions in tumor biology. It is our impression that the DA cells may be utilizing the connective tissue of the host as a vehicle for their spread.

Design: Infiltration patterns in 103 cases of resected DA were analyzed.

Results: 64 of 103 cases (62%) had vascular invasion, and in 25 (40%) of these there was a peculiar phenomenon in which the carcinoma cells lined the inner surfaces of the vessel walls, forming a well-defined duct-like structure. These were so well-formed and bland-appearing that they closely resembled normal ducts or PanIN. Actin and elastin stains were performed to confirm the intravascular nature of the duct like structures. The carcinoma cells appeared to replace the endothelial cells; CD34+ endothelial cells were

identified in the areas where carcinoma cells were detached, but none was demonstrable in areas where the carcinoma cells were adherent to the surface (carcino-endothelialized). Many of these carcino-endothelialized (duct-like transformed) vessels were identified as isolated units, far away from the main tumor, suggesting that this is an insidious form of spread by pancreatic adenocarcinoma. Similarly, in 80% of the cases with perineural invasion, carcinoma cells formed a well-defined duct like structure within the perineural spaces as well. In fact, in many cases, duct formation was more prominent in the perineural spaces than the invasive carcinoma in the regular stroma.
Conclusions: Pancreatic ductal adenocarcinoma cells show a peculiar predilection to adhere to inner surfaces of vascular channels and transform the vessels into a duct like structure by a phenomenon that we propose to refer as carcino-endothelialization. Similar process also occurs in perinueral spaces. Whether this distinctive ability of PDA cells has a role in the insidious spread and rapid progression of pancreas cancer warrants careful scrutiny. We hypothesize that that this ability of PDA cells may be related to the inherent ability of normal pancreatic ductal cells to form ducts without the support of any myoepithelial or basal cell layers (in contrast with other exocrine organs such as breast and prostate).

1487 Sox10 Expression in Neural Crest Derived Tumors

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Background: Sox10 is an important transcription factor for development of neural crest cells, which have the ability to differentiate into several cell types including melanocytes, cells of the peripheral nerve, and adrenomedullary cells. S100 protein has been the most commonly used marker for tumors of melanocytic and peripheral nerve sheath differentiation. However, this marker is also expressed in a wide variety of other types of lesions, and a more specific marker is needed.
Design: A wide variety of tumors of neural and neuroendocrine differentiation were retrieved: 77 malignant melanomas (8 in situ, 48 epithelioid, 21 spindle cell/desmoplastic), 40 peripheral nerve sheath tumors (PNSTs) (22 Schwannomas, 18 neurofibromas), 69 thyroid tumors (17 papillary carcinoma, 18 follicular adenoma, 16 follicular carcinomas, 7 insular carcinomas, 3 anaplastic carcinomas, 8 medullary carcinomas), 26 Merkel cell carcinomas, 72 lung neuroendocrine tumors (20 small cell, 9 large cell, 37 carcinoid, 6 atypical carcinoid), 19 pheochromocytomas, 23 paragangliomas, 26 adrenocortical tumors (23 adenomas, 3 carcinomas), 33 GI neuroendocrine tumors (6 gastric, 7 small intestine, 4 appendiceal, 2 colonic, 14 pancreatic). Sox10 immunostain was performed by using tissue microarrays. Common tumors and normal tissue from various organs/sites were also stained. The extent of staining was graded as 1+, 1-25%; 2+, 25-50%; 3+, 50-75%; 4+, >75%.
Results: All PNSTs showed diffuse (3+/4+) Sox10 immunoreaction. The expression was variably observed in 38 and 60% of the in situ melanoma and epithelioid invasive melanoma while it was diffusely expressed in 100% of the spindle cell melanomas. Other tumors were negative for Sox10. Only sustentacular cells demonstrated Sox10 reaction in 57, 74, 78, and 75% of lung carcinoid tumors, pheochromocytomas, paragangliomas, and appendiceal carcinoid tumors. Rare cases of other neuroendocrine tumors contained sustentacular cells. Normal tissues were negative for Sox10 except for peripheral nerve sheath, sweat glands, salivary glands, and bronchial glands.
Conclusions: Sox10 appears to be a useful marker for the diagnosis of tumors with melanocytic and Schwannian differentiation. While S100 protein remains as a sensitive stain for such tumors, Sox10 offers greater specificity. This marker seems extensively up-regulated in spindle cell melanoma, further supporting peripheral nerve differentiation in this morphologic subtype. This marker is not expressed by neuroendocrine carcinomas/carcinoid tumors of various organs, the finding consistent with non-neural crest origin but rather ectodermal origin.

1488 Micro-RNAs: A New Mechanism of Controlling Kallikrein Gene Expression in Cancer

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Background: MicroRNAs (miRNAs) are small regulatory molecules that modulate gene expression at the level of translation. Rapidly accumulating evidence has shown that microRNAs are associated with cancer. Genome-wide studies demonstrated that miRNAs are frequently located at cancer-hotspot regions, and differential miRNA expression have been documnted in cancer. Evidences suggest that miRNAs could be involved in the pathogenesis of cancer. Kallikreins are a family of genes that are shown to be dysregulated in cancer, and may serve as tumor markers. Seven kallikreins (KLK5, 6, 7, 8, 10, 11 and 14) are over-expressed in ovarian cancer. The mechanisms of dysregulation remain unclear.
Design: In order to examine the interaction between kallikreins and miRNAs, in-silico analyses using four miRNA target prediction programs were performed to identify miRNAs that target kallikreins. One miRNA, hsa-let-7f, and two of its kallikrein targets, KLK6 and KLK10, were selected for experimental verification using cell line models. hsa-let-7f was transfected into 2 cell lines (MDA-468 and HTB-161), which express high levels of KLK6 and 10. The protein levels of KLK6 and 10 at day 0, 3, 5 and 8 after transfection were assayed with ELISA. Results were compared to co-transfection with both hsa-let-7f and its inhibitor.
Results: In-silico analysis found that 86 miRNAs were predicted by more than one program as targeting KLK genes; among them, 10 were predicted to target more than one KLK. 33 (38%) of miRNAs predicted to target KLKs are differentially expressed in various cancers. A significant correlation was found between miRNAs targeting KLKs and cancer chromosomal hotspots. An miRNA that was shown to have differential expression in prostate, breast and liver cancer, *hsa-let-7f,* is strongly predicted to target both KLK6 and KLK10. Cell lines with high endogenous level of KLK6 and 10 showed a marked decrease in protein level for both kallikreins after transfection with *hsa-let-7f.* Furthermore, the decrease in protein level is rescued by co-transfection of anti-sense *hsa-let-7f.*
Conclusions: Our findings provided further support for the interaction between KLK6, KLK10 and miRNAs. Overexpression of *hsa-let-7f* in two cell lines led to a corresponding decrease in endogenous KLK6 and 10 protein level. It is possible that miRNAs have KLKs as downstream targets in cancer. Further elucidating this axis of interaction might have prognostic and/or therapeutic implications.

1489 Expression of αB-Crystallin in Human Malignant Tumors

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Background: αB-crystallin is a member of the small heat shock protein family that possess cytoprotective properties by functioning as molecular chaperones that inhibit intracellular protein aggregation. Moreover, αB-crystallin inhibits apoptosis in response to many different stimuli, including chemotherapy drugs, TNF-α and TRAIL, reactive oxygen species, and growth factor deprivation by suppressing the activation of caspase-3 and preventing mitochondrial translocation of Bax. We previously demonstrated that high levels of αB-crystallin expression are observed in a subset of breast cancers associated with poor clinical outcomes (termed basal-like). We also demonstrated that αB-crystallin contributes to the aggressive phenotype of these tumors in preclinical models. Although expression of this protein in renal cell carcinoma and malignant brain tumors has been described, few reports have examined αB-crystallin in other malignancies. The goal of the current study is to determine rates of αB-crystallin expression in a wide range of human malignant neoplasms.
Design: 543 tumors were studied using tissue microarray samples. Tumor types included carcinomas of: breast, thyroid, colon, kidney, head and neck, bladder, endometrium, liver, lung, prostate, esophagus, stomach, pancreas, cervix and ovary. Malignant brain tumors, malignant melanomas, sarcomas, testicular cancers, mesotheliomas and lymphomas were also analysed. For detection of αB-crystallin protein expression, we used a monoclonal antibody (clone SPA-222) and automated immunohistochemical methods. Greater than 5% staining was considered positive.
Results: Tumors that were found to possess detectable rates of αB-crystallin expression included: malignant brain tumors (35/36 cases), renal cell carcinomas (66/72 cases), thyroid carcinomas (31/63 cases), malignant melanomas (17/42 cases), head and neck squamous cell carcinomas (10/24 cases), ovarian carcinomas (12/65 cases) and breast carcinomas (8/64 cases). One sarcoma case was found to be positive for expression. None of the other tumor types studied were found to be positive for expression.
Conclusions: It appears that αB-crystallin protein expression can be observed in a wide variety of human malignant tumors, most of which are known to be biologically aggressive. Further studies are warranted to determine whether αB-crystallin may contribute to the pathogenesis and/or prove to be a biomarker for clinical outcomes in an expanded range of tumor types in addition to breast cancer.

1490 Gene Expression Profiling and Immunohistochemistry Define Roles for pFOXO1a and FASN in PI3K Signature in Prostate Cancer

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Background: The PI3K pathway is frequently activated in prostate cancer and occurs by multiple nonexclusive mechanisms, inactivation of PTEN being the most common. There is a lack of consensus about the true definition of the PI3K activation signature in clinical samples using phospho-antibodies. Our approach was to broaden the current definition of the pathway by integrating gene expression data and additional protein markers, in particular the Forkhead transcription factor FOXO1a and Fatty Acid Synthase (FASN), both recently shown to modulate PI3K/Akt signaling.
Design: We examined the immunohistochemical expression of key proteins in the PI3K pathway, including PTEN, pAKT (Ser 473), pS6 (Ser 240/244), pFOXO1a (Ser 256) and FASN on prostate TMAs. Paired tumor and normal (N=127) were scored for intensity and quantity of nuclear and cytoplasmic staining. For a subset of these (N=52) gene expression profiles were available. The relationship between PTEN gene expression level and each IHC marker was assessed using the Spearman coefficient. Additionally, a univariate logistic regression model was used to predict the expression levels of PTEN based on IHC scores. Spearman partial correlation was used to determine the relationship between FASN protein levels and the other proteins, after adjusting for PTEN gene expression and protein levels.
Results: Significantly only nuclear pFOXO1a was found to be associated with PTEN gene expression levels ($p=0.02$). Results from the logistic regression model using each of the IHC scores to predict the expression levels of PTEN gene confirmed that nuclear pFOXO1a is the only marker that can accurately distinguish cases with high versus low PTEN mRNA (Odds ratio 1.43, 95% CI, 0.99-2.06). FASN scores correlated with pAKT ($p=0.0008$), cytoplasmic pFOXO1a ($p<0.0001$), nuclear pFOXO1a ($p=0.05$), pS6 ($p=0.0007$). Significantly, after controlling for both PTEN gene expression and protein levels, the correlation of FASN with pAKT, cytoplasmic pFOXO1a and pS6 remained positive (partial Spearman correlation with $p<0.05$), suggesting that high FASN expression levels are associated with PI3K pathway activation independently of PTEN status.
Conclusions: Our study confirms in vivo the relationship between PI3K pathway activation and FOXO1a phosphorylation state and subcellular localization. We also demonstrate the role of FASN as an additional marker for PI3K pathway activation in cases with functional PTEN.

1491 Characterisation of Early Stemness Regulation in Teratocarcinoma Stem Cells

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Background: Extensive self-renewal and differentiation ('stemness') of cancer stem cells (CSCs), progenitor cells required for normal tissue renewal that appear likely cells of origin of tumours, may drive tumourigenesis. Furthermore, persistence of CSCs post-intervention may explain metastasis and recurrence. Identification of CSCs in brain, breast, prostate, head and neck and ovarian tumours, imply that CSCs are key players in malignancy. However, clinical inhibition of CSC stemness has not been achieved. As CSCs mirror many aspects of normal stem cells (NSCs) of comparable potency and are functionally pluripotent (can self-renew and differentiate to produce mature cell types), we hypothesised that CSCs were characterised by aberrant regulation of differentiation rather than of differentiation itself. Addressing this, we have characterised novel CSC regulatory events through analysis of self-renewal and early differentiation in CSCs.

Design: Human teratocarcinoma ('classical stem cell' gonadal tumours) CSCs originally derived from well (pluripotent) and poorly-differentiated (nullipotent) tumours were stimulated to differentiate for 3 days via addition of retinoic acid. Whole-genome array analysis was performed on undifferentiated and differentiated samples and profiles validated through further analysis of 50 genes of interest by TaqMan real-time PCR analysis. Validated gene expression profiles were bioinformatically compared to published hES data, permitting identification of gene events exclusive to CSCs.

Results: As stemness analysis has generally been conducted at approximately 1 week differentiation, we assayed CSCs at 3 days differentiation to identify early CSC stemness regulatory genes. As hypothesised, resultant data was enriched with novel regulatory genes previously unassociated with CSC stemness including regulators of stemness pathways such as Wnt, Snail, Notch and Shh and a novel marker of mesodermal differentiation in CSCs, ENO3. We hypothesise that these early stemness genes regulate key downstream stemness genes and pathways and postulate that specific CSC-targeting, in a manner not affecting NSCs, can now be achieved by their functional knockdown.

Conclusions: We have identified gene expression profiles enriched for genes involved in early cancer stemness. We believe that this new understanding of CSC biology will facilitate achievement of targeted removal of CSC-stemness in a manner applicable to cancer therapeutics.

1492 Differential Regulation of Key Stemness Genes during Early Differentiation of Teratocarcinoma Stem Cells

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Background: Novel strategies are required to further reduce rates of mortality from cancer. One such strategy involves targeting stem-like cells required for normal tissue renewal that are a likely cell of origin of tumors. Cancer stem cells (CSCs) occur in brain, breast, prostate and head and neck tumors and through extensive self-renewal and differentiation ('stemness'), may drive tumor growth. It is widely believed that CPC stemness is key in malignancy. Furthermore, persistence of CPCs post-intervention may explain metastasis and recurrence. As self-renewal and differentiation of both normal stem cells (NSCs) and CSCs of similar potency involves almost identical events, we hypothesises that it is regulation of differentiation, rather than differentiation itself, that is aberrant in CSCs. Addressing this hypothesis, we have characterised expression of key stemness events during early CSC differentiation.

Design: Human teratocarcinoma ('classical stem cell' gonadal tumours) CSCs originally derived from well (pluripotent) and poorly-differentiated (nullipotent) tumors were differentiated for 3 days-4 weeks via addition of retinoic acid. Gene expression of key stemness and cancer events was characterised by TaqMan realtime PCR analysis of marker genes.

Results: As hypothesised, regulation of the key stemness genes (Oct4-Sox2-Nanog) and pathways (Shh, Notch, Snail, Wnt) were clearly detectable in pluripotent CSCs from 3 days differentiation. While in pluripotent CSCs several pathways continued to have increasing expression from 3 days through to 4 weeks, select pathways reached their optimal level at earlier time points and remained at this level though 4 weeks. Of particular interest was the co-ordinated upregulation of several pluripotent genes in highly malignant nullipotent CSCs that cannot functionally differentiate. Furthermore, while regulation of pluripotent differentiation was believed to be absent in nullipotent cells, mesoderm differentiation was found to be coordinately regulated over time.

Conclusions: Through analysis of early differentiation gene events, we have identified early regulation of several genes and pathways that are key to CSC biology. Furthermore, we have identified key stemness genes and pathways regulated in highly malignant nullipotent CSCs. We believe that the functional knockdown of these genes may facilitate removal of stemness from CSCs in a manner applicable to cancer therapies.

1493 Prostate-Specific Membrane Antigen (PSMA) Expression in Dysplasia, Regeneration, and Repair

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Background: Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein expressed in prostate cancer and benign prostatic epithelium, as well as in endothelial cells of neovasculature from a variety of tumors. PSMA expression in non-neoplastic neovasculature has not yet been studied. To enhance our understanding of vascular proliferation and angiogenesis in non-neoplastic conditions and in events leading to fully-developed tumor associated neovasculature, we studied non-neoplastic physiologic, reparative and regenerative human tissues, as well as Barrett's mucosa with and without dysplasia, to determine the presence of PSMA-expressing neovasculature.

Design: Immunohistochemistry for PSMA using 3E6 antibody (Dako, Carpenteria CA) was applied on formalin-fixed paraffin embedded tissue from keloidal scars (n=12), granulation tissue (GT) from heart valves (n=11) and pleura (n=12), proliferative endometrium (n=10), and Barrett's mucosa (BM) without dysplasia (n=18) and with low grade (LGD) (n=12) or high grade dysplasia (HGD) (n=24). Any expression was recorded and results were scored as positive or negative.

Results: Results are listed in Table 1.

PSMA expressing vessels in regeneration, repair, and dysplasia

	Proliferative endometrium (%)	Pleural peel GT (%)	Heart valve GT (%)	Keloidal scar (%)	BM (%)	BM-LGD (%)	BM-HGD (%)
% with PSMA+ vessels	10/10 (100)	10/12 (83)	6/11 (55)	3/12 (20)	0/18 (0)	0/12 (0)	0/24 (0)

GT: granulation tissue; BM: Barrett's mucosa; LGD: low-grade dysplasia; HGD: high-grade dysplasia

Vessels of proliferative endometrium were consistently strongly positive for PSMA expression in all cases. The majority of vessels associated with GT from pleural peels and heart valves were positive. A minority of keloidal scar tissue had PSMA-expressing vasculature. PSMA was not expressed by vessels of any BM with and without dysplasia.

Conclusions: PSMA is consistently expressed in neovasculature of proliferative endometrium, a physiologic condition, as well as in a variety of non-neoplastic, regenerative and reparative neovasculature, including granulation tissue and scars. Interestingly, vessels associated with dysplastic and non-dysplastic Barrett's mucosa did not express PSMA. These findings confirm the presence of at least some shared mechanisms between vasculogenesis in neoplasia and physiologic conditions. Caution should be applied when utilizing anti-PSMA based therapies, which are already in clinical trials.

1494 Immortalization and Transformation of Human Endothelial Cells Infected with Human Herpesvirus-6

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Background: Human herpesviruses (HHV) have been reported to transform endothelial cells and fibroblasts. With the exception of HHV-8, the HHV genomes appear not to be retained in the transformed cells for long periods. Herein, we report transformation and immortalization of human umbilical vein endothelial cells (HUVEC) by HHV-6. The transformed cells contain latent HHV-6 genome and are tumorigenic.

Design: To investigate the oncogenic property of HHV-6, we have established an immortalized and transformed cell line by *in-vitro* infection of HUVEC which was xenografted to nude mice. These transformed and xenografted cells were further characterized using immunohistochemistry (IHC), molecular, and electron microscopic (EM) studies.

Results: In vitro infection of HHV-6 lead to an abortive lytic cycle and, from cells showing latent infection, immortalized cell lines have been established. They showed high proliferative activities without any endothelial growth factor requirement. Latent HHV-6 genomes were detected in an integrated form but virus production was not detected by IHC (late antigens), RT-PCR, and EM. The immortalized cell lines also expressed factor-8 (except in two cell lines in the later passages) and procoagulant activity. Xenografting in nude mice at passage 200 and beyond formed distinct tumors within 4 weeks indicating that the transformed cell lines were tumorigenic. The xenografted tumors showed epithelial rather than endothelial features including polygonal cells with occasional tubular patterns, positive pan-keratin and negative CD31, CD34, factor-8. EM confirmed epithelial and glandular features including surface microvilli, tight junctions, desmosomes with tonofilaments, cytoplasmic lumina, and intermediate filaments. No pinocytotic vescicles or weibel-palade bodies are identified.

Conclusions: Our data indicate that HHV-6 infection of HUVEC lead to latent infection with immortalization. Although, endothelial markers and pro-coagulant activities were mostly preserved in the immortalized cell lines, the loss of endothelial cell features and acquisition of epithelial characteristics were observed in the xenografts. Because HHV-6 is continuously present in the lymphocytes of sero-positive individuals and the virus is frequently reactivated in immuno-supressed individuals, HHV-6 may play an important role in the neoplastic transformation of endothelial cells during immune suppression.

1495 Automated Quantitative Assessment of Multiple Immunohistochemical Stains in Cancer Tissue Sections in Preclinical and Clinical Trials

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Background: Traditional subjective visual assessments of immunohistochemistry (IHC), while reasonably reproducible, are repetitive and provide low throughput in high-volume preclinical and clinical trials. Concordance, as measured by correlation (r), in assessment of Histoscores among pathologists trained on common control slides ranges between 0.6 and 0.95, depending on the antigen. Typical assessment rates are 100 slides per day per pathologist. Reliable assessment of more than one molecular marker per cellular compartment (nucleus, cytoplasm, membrane) is essentially impossible by visual means alone. The objective is to develop computer-based tools to improve accuracy, precision, and speed. These goals are accomplished by combining flexible machine-learning classification, cellular component segmentation, and multiplexing capabilities in a configurable automated platform. Central to this capability are the

ease and speed of algorithm training for tumor identification, appropriate workflow for batching of large numbers of images (100s to 1000s), a means for rapid review by a pathologist to flag suspect results, and databasing in a form readily incorporated into laboratory information systems.
Design: Automated assessment tools were tested on images of single chromogen-stained breast cancer samples and of multiple fluorogen-stained lung cancer samples. Concordance correlation coefficients with manual scores were calculated using the Lin method (1989). Single-stained slides were imaged with an Aperio slide scanning instrument. Multiple fluorogen-stained samples were imaged with a CRi Nuance multispectral image system. Machine learning-based segmentation algorithms were applied at reduced resolution (approximately 2X) to locate tumor, and then at full resolution (20X) to identify tumor and perform cell-compartment-based quantitation of IHC stain.
Results: Concordance coefficients between pathologists and computer-based assessments were equivalent, while the multiplexing capabilities provide functionality beyond that of visual assessment alone. Whereas manual assessments were carried out at a rate of 100 samples per day, on average among the three pathologists, automated assessments were carried out at a rate of 600-800 per day.
Conclusions: Results suggest that image analysis and multispectral imaging tools can accelerate and extend significantly automated assessment of samples in clinical and preclinical studies.

1496 The Expression Profile of 14-3-3σ Independent of p53 in 14 Major Cancer Types

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Background: 14-3-3σ is a checkpoint control gene that fosters G2 arrest and is induced by p53 after DNA damage. 14-3-3σ is also known to form a positive feedback loop with p53. Although many reports demonstrate the silencing of 14-3-3σ in several cancer types, there is no report to observe its association with p53 across various cancers in a uniform condition, to the best of our knowledge. Here, we examine the expression of 14-3-3σ and p53 in 14 major cancers, and their association and clinical significance.
Design: We immunohistochemically explored the expression of 14-3-3σ and p53 using a tissue microarray comprising 1150 cases from 14 major cancer types. The association between the expression of 14-3-3σ and p53 was examined using the chi-square test. Survival analysis was performed with the follow-up data available for patients with lung cancer.
Results: 14-3-3σ showed cytoplasmic and nuclear expression where the distribution was highly inconsistent among different cancer types. p53 nuclear staining results also varied. The frequency of the two molecules in each cancer type was not consistent, and the staining of 14-3-3σ and p53 did not show any association in all examined cancer types, regardless of normal or over expression of p53. Survival analysis examined for lung cancer cases showed no prognostic significance.
Conclusions: The expression of 14-3-3σ is extremely different from cancer to cancer where lung squamous cell carcinoma shows high levels of expression, whereas kidney and prostate cancers show almost no expression. Although 14-3-3σ is known closely related to p53 in various cancer cells, no association between p53 and 14-3-3σ was observed in any cancer types. Our results indicate the significance of p53 independent pathways to regulate 14-3-3σ expression in various cancer types. The result that 14-3-3σ did not differentiate patients' survival in lung cancer supports the proposition that the silencing of 14-3-3σ be related to tumorigenesis, but not to cancer progression.

14-3-3 σ and p53 expression in various cancers (%)

	14-3-3 σ (cytoplasm)	14-3-3 σ (nucleus)	p53 (nucleus)		14-3-3 σ (cytoplasm)	14-3-3 σ (nucleus)	p53 (nucleus)
Lung SCC	90.4	82.1	68.6	Thyroid	18.6	0.0	33.7
Urinary bladder	74.4	32.6	81.4	Ovary	18.6	16.3	48.9
Lung AD	60.3	35.8	35.5	Uterine body	13.3	6.7	46.3
Pancreas	54.3	40.0	41.2	Breast	12.3	4.9	40.7
Biliary tract	37.8	9.5	44.8	Liver	10.0	5.6	28.1
Colon	30.3	4.5	84.1	Prostate	2.6	0.0	17.0
Stomach	29.1	10.1	53.1	Kidney	1.1	0.0	6.1

1497 Identification of Human Tumor Stem Cells by a Novel Detection Strategy Based on Comparative Genomic FISH

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Background: The existence of stem cells in human solid tumors has been inferred by dilutional clonality and marker studies *in vitro* and clinical observations of tumor latency, recurrences and drug resistance *in vivo*. We, in fact, have studied the existence of stem cells in a human model of inflammatory breast cancer termed MARY-X, a model that forms spheroids which are similar to the embryonal blastocyst. Comparing MARY-X with common non-IBC breast carcinoma and normal cell lines, we found specific markers (Stellar, H19, Rex-1, Nestin, CD133) known to be associated with embryonal stem cells present within the MARY-X spheroids. RT-PCR analyses of MARY-X also revealed the expression of OCT4, SOX2, and Nanog, transcriptional determinants essential for the pluripotency and self-renewal of human embryonal stem cells. However these findings alone do not prove that there is a true hierarchical reserve stem cell compartment since both stem cell markers and functions could be transiently and reversibly derived from the proliferating tumor cell populations.
Design: We reasoned that true hierarchical stem cells should have fewer genomic alterations (gains and losses) than the major proliferating population of the tumor. To this end we conducted array CGH of MARY-X and identified 10 regions of amplification and 10 regions of loss. We then derived probes from each of these regions and conducted multicolor FISH on individual cells of MARY-X disadhered from the spheroids.
Results: Each of the probes recognized a specific chromosomal region with both metaphase and interphase FISH of normal fibroblasts. All of the probes derived from regions showing gains by array CGH also showed marked amplification (5-20 fold) in the vast majority of the MARY-X cells (>99%). All the probes derived from regions showing losses showed decreases or complete absences in the vast majority of cells. However there was a distinct subpopulation of MARY-X cells (<1%) that contained fewer genomic alterations (fewer losses and fewer gains) by multicolor FISH. This more "genomically stable" minority subpopulation of cells expressed increased notch signaling compared to the "genomically unstable" majority population.
Conclusions: The existence of a minority subpopulation of cells of MARY-X that exhibit greater genomic stability coupled with the observation that they exhibit increased notch signaling, a signaling pathway thought be activated in embryonal and tumor stem cells, is evidence that a hierarchical stem cell population exists in solid cancers.

1498 Suppression of Ovarian Cancer Metastatic Colonization by MKK4 Is Associated with Cellular Growth Arrest and Upregulation of p21

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Background: A longstanding question in metastasis research is why some disseminated cancer cells fail to complete steps of metastatic colonization for extended periods of time. Our laboratory identified Mitogen-Activated Protein Kinase (MAPK) Kinase 4 (MKK4) as a metastasis suppressor protein in a mouse xenograft model of intraperitoneal (IP) ovarian cancer metastasis. In this model, expression of MKK4, via activation of p38, delays metastasis formation and prolongs animal survival. Here, we elucidate the kinetics of this delay as well as the biological mechanisms underpinning it.
Design: Athymic nude mice were injected IP with SKOV3ip.1 ovarian cancer cells stably expressing vector or MKK4 and sacrificed from 3 to 80 days post injection (dpi). qRTPCR was used to quantitate cells adherent to the omentum at 3 dpi. TUNEL reaction, BrdU incorporation, and pH3, p21, and p27 expression were scored by immunohistochemistry in micrometastases at 14 dpi. Kinase assays and immunoblots were performed on cell lines derived from macroscopic MKK4-expressing metastases.
Results: Using the Gompertz function to model the kinetics of metastasis, we found that the rate of accumulation of macroscopic metastases is significantly decreased for MKK4-expressing cells. MKK4 expression did not have a substantial effect on the number of cells initially adhering to the omentum, nor was the rate of apoptosis increased in these cells. Instead, MKK4-expressing cells fail to proliferate once they reach the omentum and significantly upregulate p21, a cell cycle inhibitory protein. Consistent with the kinetic modeling, *in vitro* kinase assays and *in vivo* passage of cell lines derived from macroscopic metastases demonstrates that eventual outgrowth of MKK4-expressing cells is not due to selection. Rather, the population of MKK4-expressing cells eventually downregulates p21 expression and homogeneously adapts to the consequences of upregulated MAPK signaling.
Conclusions: It is unclear why some disseminated cancer cells fail to proliferate, while others break dormancy and complete the process of metastatic colonization. Our data support a model in which cancer cell growth is controlled by the bidirectional crosstalk between disseminated cancer cells and their microenvironment.

1499 In Situ Carcinomas Can Exhibit Vasculogenic Mimicry

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Background: Human tumor progression is thought to involve the sequential steps of *in situ* carcinoma, stromal invasion and lymphovascular invasion (LVI). Recent experimental evidence has suggested that carcinomas may short-circuit this process by inducing vasculogenic mimicry in which either tumor or mesenchymal stem cells directly differentiate into blood vessels. We wondered whether we might find evidence of this phenomenon in human *in situ* carcinomas where initial observational studies indicated that areas of *in situ* carcinoma were seen directly juxtaposed to areas of LVI without any intervening stromal invasion.
Design: We decide to investigate 10 cases of breast ductal carcinomas in situ (DCIS) where areas of LVI were observed adjacent to areas of DCIS. We examined these cases with both morphometry as well as IHC. Our IHC studies utilized proliferation (Ki-67), tumor (E-cadherin), myoepithelial (p63), lymphatic (D2-40) and vascular (CD31) markers singly and in combination, the latter employing a dual chromogen technique.
Results: The DCIS clusters exhibited identical proliferation, E-cadherin immunoreactivity and cross-sectional area as the tumor emboli within either lymphatics or blood vessels ($p > .5$). The DCIS clusters exhibited a progressive loss (50%-100%) of p63 myoepithelial immunoreactivity. Four adjacent vascular populations were in evidence: D2-40 lymphatics with and without tumor emboli; CD31 blood vessel capillaries with and without tumor emboli. The D2-40 lymphatics containing tumor emboli showed focal evidence of residual p63 whereas the D2-40 lymphatics without tumor emboli were negative. Similarly the CD31 blood vessels containing tumor emboli showed focal evidence of residual p63 whereas the CD31 vessels without tumor emboli were also negative. The lymphovascular channels containing tumor emboli exhibited a weaker and less circumferential pattern of D2-40 or CD31 immunoreactivity compared to the channels devoid of emboli.
Conclusions: The identical size, proliferation index and E-cadherin immunoreactivity of the DCIS clusters and the LVI tumor emboli suggest that these structures are one and the same and remain intact during their DCIS to LVI transition. The dual p63/D2-40 and p63/CD31 immunoreactivities in the lymphovascular channels containing emboli and their relatively immature pattern of D2-40/CD31 staining suggest that they represent

newly created vasculature derived from myoepithelial lined ducts. Collectively this evidence supports the hypothesis that *in situ* carcinomas can exhibit vasculogenic mimicry and metastasize without invading.

1500 Genomic Map of Bladder Cancer Development

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Background: Maps of genomic imbalances can facilitate the search for genes and genomic sequences involved in the development of cancer. The identification of those chromosomal regions that provide growth advantage for occult *in situ* preneoplastic conditions progressing to invasive cancer is of particular importance. We report on the construction of genome-wide map of human bladder cancer that tracks its progression from *in situ* precursor conditions to invasive disease.

Design: Testing for allelic losses across 787 microsatellite markers mapped to chromosomes 1-22 was performed on multiple DNA samples extracted from the entire mucosal surface of the bladder and corresponding to normal urothelium, *in situ* preneoplastic lesions, and invasive carcinoma. We also performed high resolution mapping with single nucleotide polymorphic sites (SNPs) markers of one of the critical chromosomal regions that contain a model tumor suppressor gene, RB1 and defined a minimal deleted region associated with clonal expansion of *in situ* neoplasia.

Results: By analyzing genome-wide patterns of allelic losses, we found three major waves of genetic changes associated with growth advantage of successive clones reflecting a stepwise conversion of normal urothelial cells into cancer cells by a process analogous to Darwinian evolution and identified six regions of allelic losses mapping to 3q22-q24, 5q22-q33, 9q21-q22, 10q26, 13q14, and 17p13 that may represent critical hits driving the development of bladder cancer. By analyzing the genomic content of the minimal deleted region around RB1, we provided new insights on the involvement of several non-coding sequences and identified novel target genes termed forerunner genes involved in early phases of cancer development.

Conclusions: Our map depicts the evolution of genome-wide allelic losses in the development of a common human cancer and provides a global look at genome involvement in carcinogenesis.

1501 Profiling the Expression of PTEN, mTOR, and HIF-1 in Prostatic Neoplasia

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Background: Phospho-mTOR (p-mTOR)(Ser2448) is a component in the PTEN/PI3K pathway. Hypoxia-inducible factor-1(HIF-1), a transcription factor is upregulated in hypoxia. P-mTOR regulates translation of HIF-1mRNA and HIF-1suppresses p-mTOR activity. Deletion of PTEN i sa negative regulator of mTOR. The immunohistochemical (IHC) expression of p-mTOR and HIF-1in benign (BN) prostate, PIN (prostatic intraepithelial neoplasia) and prostate cancer (PCa) was investigated. Frequency of PTEN deletion in Gleason (GL) 6, GL7 and high grade (HG) (GL8 and 9) PCa tissue microarrays (TMAs) was investigated by FISH.

Design: TMAs were constructed from 35 prostatectomies for each of: PIN, GL 6, GL 7 and HG and IHC was performed on a TMA from each group for p-mTOR and HIF-1. P-mTOR was also evaluated in 20 BN prostate biopsies. The intensity of IHC staining was recorded and the percent positive glands was scored. The cases were analyzed by dual-color FISH with commercial DNA probes for the PTEN locus (band 10q23) and the centromere for chromosome 10 (band 10p11.1-q11.1).

Results: HIF-1expression significantly increased with increasing GL score. HIF-1staining in PIN was 9%, Gleason 6: 46%, GL 7: 54% and HG: 69% ($p<0.0001$). P-mTOR expression from BN to HG tumors showed a significant negative correlation with increasing GL score. The median p-mTOR percent expression was 75% for PIN, 51% for GL 6, 4 % for GL 7 and 11% for HG ($p<0.0001$). In PIN, GL6 and GL7, the percent p-mTOR tended to be larger in cases positive for HIF-1(37% vs. 51% respectively). This was reversed for HG tumours (median p-mTOR in HIF-1positive was 8% and HIF-1negative was 37%). Frequency of PTEN deletions increased significantly with increasing GL score ($p=0.04$).

Conclusions: Weak p-mTOR expression was observed in BN tissue, strong in PIN and GL6 only, no expression in GL7 or higher. The p-mTOR expression in PCa was significantly less than in BN. In PIN, GL 6 and HG, p-mTOR expression may be elevated with PTEN deletion. HIF-1 expression increases with increasing GL score. P-mTOR expression may be sensitive to hypoxia through negative feedback by HIF-1. The P-mTOR feedback inhibition by HIF-1 correlates with decreased or absent expression of p-mTOR with increasing GL score.

1502 Immunophenotypic Differences of Tumor-Infiltrating T Cells in Subtypes of B Cell Lymphoma

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Background: B cell lymphomas often contain numerous tumor-infiltrating T cells (TILs). However, it is not known whether T cell subsets from different B cell lymphoma subtypes are similar. As a first step in examining the role of TILs in B cell lymphoma, we have compared the flow immunophenotype of TILs in 114 cases of pretreated B cell lymphoma.

Design: Flow cytometry results from 114 cases of pre-treated B cell lymphoma collected at UTMB from 1999-2007 were examined. Cases included 48 follicular lymphomas (FL), 28 diffuse large B cell lymphomas (DLBCL), 19 small lymphocytic lymphomas (SLL), 9 marginal cell lymphomas (MRGL), 7 mantle cell lymphomas (MCL), and 3 Burkitt lymphomas (BL). Parameters examined included CD3+ T cells, CD4+ T cells, CD8+ T cells, CD3+CD16/56+ NKT cells, CD3-CD16/56+ NK cells, and CD4/CD8 ratio. Statistical signficance of results were determined with the Kruskal-Wallis one-way ANOVA and Bonferroni all-pairwise multiple comparison tests.

Results: The highest CD3 T cell content was noted in FL (34%) and MRGL (39% NS), and the lowest in SLL (19%) and MCL (16% NS). The highest CD4 T cell content was noted in FL (23%) and the lowest in MCL (8.1%), DLBCL (12%), and SLL (12%). The highest CD8 T cell content was noted in DLBCL (14%) and BL (13% NS), and the lowest in SLL (3.8%). The highest CD4/CD8 ratio was noted in SLL (3.4) and FL (3.2), and the lowest in DLBCL (1.2%) and BL (0.72% NS). The highest NKT and NK cell counts were noted in DLBCL (2.4%, 2.6%) and BL (2.5% NS, 3.1% NS), and the lowest in SLL (0.55%, 0.58%) and FL (1.0%, 0.86%). All results above except those marked NS were highly statistically significant ($p<.002$) (see table 1).

T CELL SUBSETS IN B CELL LYMPHOMA

	DLBCL	FOLLICULAR	SLL	MARGINAL	MANTLE	BURKITT
CD3	28	34 !	19 *	39	16	24
CD4	12 *	23 !	12 *	21	8.1 *	11
CD8	14 !	9.7	3.8 *	9.9	7.3	13
CD4/CD8	1.2 *	3.2 !	3.4 !	2.6	1.3	0.72
NKT	2.4 !	1.0 *	0.55 *	2.0	0.53	2.5
NK	2.6 !	0.86 *	0.58 *	0.98	0.76 *	3.1

Statistically significant differences are marked by ! (high value) and * (low value). Cell subsets are expressed as mean % and CD4/CD8 ratios are expressed as mean decimals.

Conclusions: Follicular and marginal lymphoma are marked by numerous infiltrating T cells, mostly CD4 cells. In contrast, diffuse large and Burkitt lymphoma contain more CD8+ T, NKT, and NK cells. Small lymphocytic and mantle lymphoma, by contrast, contain fewer T cells, NKT, and NK cells. These results suggest that potentially tumor-toxic T cells are recruited to DLBCL and Burkitt tumors, but not to other B cell lymphomas.

1503 "Funnel Factors" in Human Cancer as Key Functional Factors in Cell Transformation: A New Paradigm of Pronostic Signatures in Human Tumors

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Background: Malignant cell transformation requires the accumulation of many genetic alterations which affect the patways of cell sufficiency in cell growth, apoptosis, cell cycle, invasiveness and metastasis. In each main cellular pathway there are many genes and factors that can be involved. Cell signaling pathways include a complex myriad of interconnected factors from the membrane to the nucleus, such as erbB family receptors and the PI3·K/Akt/mTOR, Ras-Raf-ERK cascades and 4E-BP1 and the EIF family of factors which control protein synthesis in ribosomes In an attempt to identify molecules that clearly reflect the oncogenic role of cell signaling pathways in human tumors, we propose a concept we term "funnel" factor, a factor where several oncogenic signals converge and drive the proliferative signal downstream to the ribosomes or the nucleus.

Design: To find pivotal or funnel factors in the cell signalling pathway, we studied the expression of several factors involved in cell signaling in 110 ovarian tumors, 210 breast carcinomas, 90 prostate tumors, 75 gliomas, 130 endometrial tumors and 120 colon carcinomas by immunohistochemistry and Western blotting. The factors studied were: Her1 and Her2 growth factor receptors, the RAS-RAF-MAPK and the PI3K-AKT-mTOR pathways and the downstream factors p70, S6, 4E-BP1, and EIF4E. Normal (IMR90) and colon and breast cancer cell lines (MDA-MB-231,MDA-MB-435, HCT116p53- and HCT11653+) were also studied.

Results: We found that phosphorylated 4E-BP1 expression in breast, ovary, endometrial, gliomas and prostate tumors was associated with malignant progression and an adverse prognosis, regardless of the upstream oncogenic alterations. In the in vitro studies, transfection in those cell lines of 4E-BP1 with phosphorylation site mutations suppressed cell proliferation.

Conclusions: With these results, p-4E-BP1 seems to act as a funnel factor for an essential oncogenic capability of tumor cells, self-sufficiency in growth signals, and could be a highly relevant molecular marker of malignant potential. Further investigation into this concept may identify additional funnel factors in this and others oncogenic pathways and provide potential therapeutic targets.

1504 Quantitation of TMEM (Tumor Microenvironment of Metastasis) in Human Primary Breast Cancer Samples Is Predictive of Long-Term Metastatic Potential

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Background: Multiphoton-based intravital imaging (IVI) has demonstrated that carcinoma cells in mouse and rat mammary tumors exhibit increased motility and intravasation when associated with perivascular macrophages, resulting in increased hematogenous metastasis. The invasive tumor cells interacting with macrophages in vivo exhibit a unique expression profile called the invasion signature. Mena is a key master gene in the invasion signature and is a marker for invasive tumor cells. Using an IHC triple stain for mena-expressing tumor cells, vessels (CD31), and macrophages (CD68), we have identified this microanatomic landmark in human breast tumors, which we call TMEM. In the evaluation of an initial series of invasive tumors, TMEM counts significantly increased with tumor grade, and the differences were sharpest if the tumors were divided into two groups: well differentiated and moderately/poorly differentiated. The purpose of this study was to assess TMEM counts in human breast cancers with known outcomes.

Design: The Cancer Registry of WCMC was searched for pairs of breast cancer cases matched for histologic grade, size, LN status, ER/PR status, and follow up time, differing

only in the presence or absence of metastatic disease. Thirty case pairs with either moderate or poor differentiation were identified. Each of the cases was stained with the IHC triple stain described above, and 20 digital images at 400X were taken of each case. Numbers of TMEM, defined as the tripartite arrangement of a macrophage and a mena-expressing tumor cell no more than 1 cell diameter from each other and from a blood vessel, were determined for each case. Cases were evaluated by two pathologists, both of whom were blinded to the clinical outcome. TMEM counts for the cases and their matched controls were analyzed using a paired t-test.

Results: To date, 8 pairs have been evaluated. The average TMEM count for each group is shown below ($p<0.05$).

TMEM Count

	Metastatic cases	Non-metastatic cases	
No. of TMEM	45±18	29±19	p<0.05

Values given are mean±standard deviation

Conclusions: Preliminary analysis suggests that TMEM quantitation in primary breast cancer samples is an independent predictor of long-term metastatic outcome. Analysis of the remaining 22 pairs is ongoing.

1505 Cancer Tissue Proteomics by Heparin Affinity Fractionation Enrichment (HAFE): Up-Regulation of PSB7 and PRDX1 in Colorectal Adenocarcinoma

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Background: Colorectal adenocarcinoma is one of the worldwide leading causes of cancer deaths. Therefore, early detection of colorectal cancer progression and the identification of underlying pathogenetic mechanisms are important tasks. Global proteomic approaches have thus far been limited by the very large dynamic range of molecule concentrations in tissues.

Design: Paired cancerous and normal clinical tissue specimens from patients with colorectal adenocarcinomas were studied by heparin affinity fractionation enrichment (HAFE) followed by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and tandem mass spectrometric (MS/MS) identification.

Results: 32 proteins were found to be differentially expressed with >2-fold changes in abundance. MS/MS was used to identify 5 selected differentially expressed proteins as proteasome subunit β type 7 (PSB7), hemoglobin α subunit (HBA), peroxiredoxin-1 (PRDX1), argininosuccinate synthase (ASSY), and signal recognition particle 9 kDa protein (SRP09). The relative spcificity of PSB7 and PRDX1 overexpression in colon cancer was validated by Western blot analysis of 9 patients with colon adenocarcinomas and comparison with 10 patients with lung adenocarcinomas. Furthermore, immunohistochemistry on tissue sections was used to localize protein overexpression within heterogeneous tumor tissue to the cytoplasmic and nuclear (PSB7) and cytoplasmic (PRDX1) compartments of the neoplastic colon cancer cells.

Conclusions: This is the first proteomic study detecting differential expression of these proteins in human colorectal cancer tissues. We discuss the functional implications of PSB7 and PRDX1 protein up-regulation and explore the power of tissue proteomics for the discovery of disease biology. Furthermore, we show that heparin affinity fractionation enrichment (HAFE) is a powerful and broadly applicable new tool for interrogating the low-abundance human tissue proteome.

1506 Precancerous Stem Cells Can Be Progenitors for Tumor Vasculogenesis

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Background: The theory of tumor neovascularization has been attributed to angiogenesis, a process of forming new blood vessels from existing ones. Increasing data suggest that tumor neovascularization may be mainly resulted from tumor vasculogenesis, a process of forming new blood vessels from tumor-derived progenitor cells. Recently, we have found that precancerous stem cells (pCSCs), which represent early stage of developing cancer stem cells (CSCs), have the potential to differentiate into endothelial-like cells. Thus, we hypothesize that pCSCs can serve as progenitors for tumor vasculogenesis.

Design: eGFP-expressing murine pCSC lines were generated and transplanted into SCID CB17 mice. The pCSCs-derived lymphomas were analyzed for $eGFP^+$ blood vessels using fluorescent microscope. The vasculogenic capacity of pCSCs was tested under cytokine-conditioned or hypoxic culture condition, and compared with the monocytic tumor cells (MTCs) derived from the same mice. To verify the finding from animal tumors, human xenograft lymphomas as well as native breast and cervical cancers were analyzed for tumor cell-derived blood vessels by identification of human endothelial cell or progentior markers (CD45, CD31, CD34 and vWF) using immunohistochemical staining.

Results: We report that in the pCSC-derived tumors, most blood vessels were derived from pCSCs. ~5% pCSCs constitutively expressed vasculogenic receptor VEGFR2, which can be up-regulated by hypoxia and angiogenesis-promoting cytokines, including GM-CSF, Flt3 ligand, and IL-13. The GM-CSF-induced VEGFR2 expression was completely inhibited by IL-4. The pCSCs are much more potent in tumor vasculogenesis than the differentiated MTCs from the same tumor, which had comparable or even higher capacity to produce vascular growth factors. The results suggest that the vasculogenic capacity of pCSCs is associated with their intrinsic stem-like property. Consistently, the tumor vasculogenesis was also observed in human cancers such as cervical cancer and breast cancer and xenograft lymphoma. The tumor cell-derived endothelial-like cells had highly variable phenotype compared to endothelial cell markers found on normal endothelial cells.

Conclusions: Our studies for the first time directly prove that pCSCs can serve as the progenitors for tumor vasculogenesis, and may explain why anti-angiogenic cancer therapy trials are facing challenging.

1507 CD87, a Novel Flow Cytometry Marker in Diagnosing Myelodysplastic Syndrome

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Background: Flow cytometry has been shown to detect aberrant phenotypic changes that can aid in the diagnosis of myelodysplastic syndrome (MDS). Such changes include an altered CD13/CD16 expression pattern, decreased CD10/CD16 expression and aberrant expression of CD34 and HLA-DR on maturing myeloid cells. CD87 (urokinase plasminogen activator receptor) is expressed at the late band and segmented stage of myeloid maturation. In the study, we evaluated the clinical usefulness of decreased expression of CD87 in the diagnosis of MDS.

Design: We performed a retrospective chart review of 40 patients between 2006 and 2007 who had a flow cytometry panel, including CD13, CD16, CD10, CD87, HLA-DR and CD34 antibodies, performed for the clinical suspicion for MDS. Cases were considered positive by flow cytometry if they demonstrated at lease two aberrant phenotypic changes described above. In addition, cytogenetic and bone marrow biopsy results were compiled.

Results: Of the 40 patients (21 male vs 19 female, mean age 68 years, range 37-98) who had flow cytometry performed, 16 patients showed features of MDS on bone marrow biopsy and five patients had cytogenetic abnormalities consistent with MDS. There was a significant decrease in the expression of CD87 in patients with a positive bone marrow biopsy when compared to patients with a negative biopsy (25% vs. 50% expression, respectively, $p<0.01$). The sensitivity and specificity of the entire flow cytometry panel (CD13, CD16, CD10, CD87, HLA-DR and CD34) was 75% and 88%, respectively.

Conclusions: Flow cytometry is a sensitive assay which can be used to diagnose MDS, in conjunction with cytogenetic and histologic studies. Additionally, CD87 is a myeloid marker which may increase sensitivity and specificity when added to an MDS flow cytometry panel.

1508 Profiling the Expression Pattern of GPI Transamidase Complex Subunits in Human Cancer

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Background: The GPI transamidase complex (GPIT) consists of five subunits: PIG-U, PIG-T, GPAA1, PIG-S and GPI8 and plays a crucial role in the ultimate step of attaching GPI anchors to target proteins. Based on our previous reports incriminating PIG-U as an oncogene in bladder cancer and PIG-T and GPAA1 as oncogenes in breast cancer, we attempted to evaluate the expression pattern of the GPIT subunits in human tumors from various anatomical sites.

Design: c-DNA expression status of *PIG-U/PIG-T/GPAA1/GPI8/PIG-S* in various cancers was investigated using cancer profiling array II having c-DNA spots from 19 different organs comprising 154 tumor and corresponding normal tissue from individual patients (Clontech Laboratories, Mountain View, CA). Polyclonal antibodies against all five subunits of GPIT complex i.e. PIG-U/PIG-T/GPAA1/GPI8/PIG-S were either custom synthesized or procured if commercially available. High density multiple organ cancer and normal tissue microarrays (TMA) were purchased from US Biomax Inc. (Rockville, MD). The array had cores of 500 cores from 15 most common cancer types (20-35 cases/type) along with normal controls. All immunohistochemisty stains were evaluated by two pathologists. Four tear Intensity score was assigned per case. Data were grouped into two categories: positive (strong, intermediate) and negative (weak, null). Statistical analyses were done using STATA Statistical Software: Release 9.0.

Results: In general, we found more frequent expression of GPIT subunits in cancerous than in normal tissues. Among 19 anatomic sites analyzed, breast, ovary and uterus showed a dramatic and clear overexpression trend (in 20-70% of cases). The GPIT subunits seem to be relevant clinical markers in ovarian and lymph node malignancies. The expression of PIG-T, GPAA1 and GPI8 was absent in 40%, 80% and 75% respectively of non-malignant ovarian tissues. Moreover, none of the normal lymph node tissues showed expression of PIG-U and GPI8.

Conclusions: GPIT complex subunits are ubiquitously expressed in many tumor types. These results extend our previous findings that PIG-U, PIG-T and GPAA1 function as oncogenes and are primarily connected to tumor progression in specific tissues.Also, to the best of our knowledge, this is the first report showing the involvement of GPI8 and PIG-S in cancer progression.The clinical significance of these proteins as cancer markers and their potential role in prognosis and therapeutic intervention need to be further evaluated.

1509 Clues to the Stem Cell Origin of Human Cancers by Studying a Registry of Organ Transplant Recipients Who Later Developed Secondary Solid Cancers

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Background: The existence of stem cells in human solid cancers has been inferred by dilutional clonality and stem cell marker studies *in vitro* yet this evidence is indirect. Because of anecdotal reports of tumors of donor origin arising in transplant recipients and because this observation, if confirmed, would provide direct evidence for a stem cell origin of solid cancers, we decided to study a registry of human transplant recipients who later developed secondary solid cancers to investigate this question.

Design: We studied 115 renal, 15 heart, 25 liver, 4 lung, 25 bone marrow and 10 stem cell transplant patients. The solid cancers arising in these patients included skin, soft tissue, lung, liver, kidney, breast and others. Approximately 40% of these transplants were from different sex donors. We created a tissue microarray (TMA) consisting of the secondary solid cancers, adjacent normal tissues and cancers arising in non-transplant patients. We conducted X and Y chromosome FISH on this TMA. In selected cases

we supplemented our FISH studies with microsatellite marker studies which could distinguish donor from recipient and ploidy and gene rearrangement studies to exclude donor-recipient chimerism.
Results: Approximately 10% of the secondary solid cancers arising in non-sex matched transplant recipients were of donor origin. The vast majority of these were in patients receiving a bone marrow transplant. This was seen in both female as well as male recipients. These numbers may actually have been higher than observed because some cancers of male origin spontaneously lost the Y chromosome. This observation not withstanding, cancers with the Y chromosome occurring in females and cancers with XX signals in males indicated their donor origin. In donor organs where some cancers developed, the cancers were recipient in origin in 5% of the cases. In selected cases, microsatellite marker studies confirmed the donor or recipient origin. Ploidy and gene rearrangement studies excluded lymphocytic fusion as a mechanism to explain the findings.
Conclusions: Our studies suggest that solid cancers arising in transplant recipients can take origin from stem cells that are derived from either the transplanted organ (usually bone marrow) or the host. In either case these stem cells do not initially reside in the organ where the cancer develops. These observations could explain such tumoral phenomena as the rareness of clonal transformation, dormancy, local recurrences and distal metastases.

1510 Major Mitochondrial DNA Deletions in Obese Transgenic Mice Overexpressing Human 8-Oxoguanine DNA Glycosylase 1 (hOGG1) Gene: A Causal Relationship between Mitochondrial DNA Damages and Obesity

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Background: Mitochondria are dynamic organelles involved in oxidative phosphorylation and production of reactive oxygen species (ROS). Increasing evidence supports that mitochondrial DNA damage and dysfunction play vital roles in the development of a wide array of mitochondria-related diseases, such as obesity, diabetes, infertility, neurodegenerative disorders and malignant tumors in human. We previously described the development of obesity in transgenic (TG) mice overexpressing human 8-oxoguanine DNA Glycosylase 1 (hOGG1). We now reported here that major mitochondrial DNA deletions are frequently found in a variety of organs in these hOGG1 TG mice.
Design: our laboratory previously produced obese hOGG1 TG mice. Mitochondrial DNA samples were extracted from the liver, brain, skeletal muscle and brown fat of hOGG1 TG and non-TG control mice and subjected to PCR reaction using 8 primer sets franking the breakpoint of 7 major mitochondrial DNA deletions. Six deletions (3.7, 3.82, 3.86, 4.2, 4.9 and 5.2 kilobase in length) have been previously reported in aged mice. One novel deletion of 15.kilobase was identified in hOGG1 TG mouse in our lab.
Results: Among 7 major mitochondrial DNA deletion analyzed, 5 (3.7, 3.86, 4.2, 5.2 and 15 kilobase in length) were detected abundantly in various organs of hOGG1 TG but not in non-TG control mice. All these 5 deletions were seen in brown fat of hOGG1 TG mice. Two deletions (3.82 and 4.9 kilobase in length) were seen in both TG and non-TG mice, indicating that. These 2 deletions may not be biologically significant. Accordingly, protein expression of major mitochondrial complexes were significantly reduced in hOGG1 TG mice as compared to those of non-TG control mice.
Conclusions: major mitochondrial DNA deletions with resultant reduced mitochondrial protein expression may represent an important molecular mechanism by which these hOGG TG mice develop obesity, infertility and malignancy.

1511 Syndecan-1 Expressing Mammary Fibroblasts Produce an Extracellular Matrix Which Promotes Breast Carcinoma Cell Invasion and Directional Movement

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Background: Induction of syndecan-1 (SDC1) in stromal fibroblasts of breast carcinomas stimulates breast carcinoma growth via shed SDC1 ectodomain (Cancer Res 64 (2):612-621, 2004; J Biol Chem 282(20):14906-15, 2007). In the present study, we tested the hypothesis that the extracellular matrix (ECM) produced by SDC1-positive fibroblasts also participates in breast carcinoma progression.
Design: 3-D ECMs were generated by alkaline detergent extraction of overconfluent cultures of SDC1-positive and negative immortalized human mammary fibroblasts. After cell removal, the ECM preparations (ECM-SDC1 and ECM-mock) remained attached to the culture vessels and did not contain detectable levels of residual SDC1. T47D and MDA-MB-231 breast carcinoma cells were seeded onto the 3-D ECMs to observe carcinoma cell behavior dependent on ECM type.
Results: ECM-SDC1 showed a parallel fiber architecture characteristic of carcinoma-associated ECM and contrasted with the haphazard fiber arrangement of ECM-mock. Compared to ECM-mock, ECM-SDC1 promoted breast carcinoma cell attachment, invasion and directional movement but not proliferation. To determine whether the parallel fiber architecture was causally involved in the directional movement of carcinoma cells, ECM producing fibroblasts were seeded on different fibronectin patterns produced by microstamping. When grown on fibronectin lines (as opposed to squares), mock-transfected fibroblasts produced ECM with a parallel fiber architecture, mimicking the architecture seen in ECM-SDC1. Migration of carcinoma cells in these matrices was directional, similar to their migration in ECM-SDC1.
Conclusions: SDC1 expressing mammary fibroblasts, which are found in the desmoplastic stroma of breast carcinomas, produce an ECM with a parallel fiber architecture, which is permissive to breast carcinoma cell directional migration and invasion.

1512 The Expression Pattern of Myocyte Enhancer Factor (MEF2) Isoforms in Brain Glial Tumor

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Background: Recent studies showed that MEF2 isoforms might play a role in neuronal maturation during the CNS development. In situ hybridization with nucleic acid probes showed that mRNA of mouse MEF2 isoforms were present in different brain compartments with varied levels at different embryonal and postnatal ages. Immunohistochemical study with a non-isoform specific MEF2 antibody showed the MEF2 proteins are localized in most neuron in mouse cerebellum. Overexpressions of MEF2A and 2D have been shown to specifically promote differentiation and survival of cerebellar granule cells, however, it is not clear if MEF2s are expressed in neurogenic tumors. In this study, we explored the expression patterns of MEF2 isoforms in glial tumors with different WHO grades by using immunohistochemistry with isoform-specific MEF2 antibodies.
Design: Glioblastoma multiforme (6), anaplastic astrocytoma (3), pilocytic astrocytoma (6), subependymal giant cell astrocytoma (SEGA) and oligodendroglioma (6) cases were reviewed. Three MEF2 isoform specific antibodies (MEF2A, MEF2C and MEF2D) were used in the study. The specificity of antibodies had been documented by Western blot with in vitro overexpressed MEF2 isoform proteins and Gel shift assay (Zhu and Gulick: Mol. Cell. Biol. 2004, 24:8264-8275). Immunostaining with MEF2 isoform-specific antibodies and isoform non-specific antibody (Rabbit polyclonal antibody, Santa Cruz, CA) were performed with proper positive and negative controls. The stain results were assessed by two pathologists and graded as positive (++), weak positive (+) and negative (-).
Results: All the MEF2 isoform-specific and non-specific antibodies stained the nuclei in the control and positive cases. There is no cytoplasmic expression identified. The positive stained cells including tumor cells, astrocytes and neurons in adjacent brain tissue were distributed in scattered pattern. MEF2s are expressed in all the astrocytomas except SEGA. The express pattern of MEF2 isoforms are listed in the Table:

Table

	MEF2A	MEF2C	MEF2D	MEF2s
GBM	0/6	2/6	6/6	6/6
Anaplastic	0/3	2/3	2/3	3/3
Pilocytic	0/6	4/6	5/6	6/6
SEGA	0/2	0/2	0/2	0/2
Oligodendroglioma	0/6	4/6	3/6	6/6

Conclusions: MEF2 proteins are irregularly expressed in glial tumors of different grades, indicating that histologic grade does not alter MEF2 expression patterns, however, the isoform expression patterns are different. MEF2A isoforms are undetectable in glial tumors while the positive rate for MEF2D in glial tumors is higher than that for MEF2C.

1513 Histological Detection of Human Papilloma Virus High Risk Types in Sinonasal Inverted Papillomas by In Situ Hybridization

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Background: The etiology of sinonasal inverted papilloma (IP) is considered to be associated with Human papilloma virus (HPV). HPV has been genotyped or probed by PCR, "tissue" in-situ hybridization (ISH) and Southern blot. Correlative morphological viral detection of HPV high risk (HR) types in IP has not been reported. We performed histological HPV ISH on 21 sinonasal IP and demonstrated HPV HR types corresponding to the IP cellular neoplastic changes.
Design: Twenty-one excisional biopsy specimens of the nasal cavity and sinuses were obtained from 17 patients over a three year period (2005-2007) from 2 tertiary medical centers. Patients included 12 male and 5 female, ranging in age from 36 to 72 years (average 56 years). The extent of dysplasia was based upon histology. Automated HPV ISH analysis was performed for the hybridization on 4µm thick formalin fixed and paraffin embedded tissue sections, using a Bench Mark XT (Ventana Medical Systems, Tucson, AZ) with DNA probes for HPV HR (types 16,18,31,33,35,39,51,52,56,58 and 66). Positive and negative controls were hybridized alongside the study cases. Positive staining was visualized using the precipitating chromogenic reaction NBT/BCIP with a nuclear localization.
Results: HPV HR types were detected in 6 of 21 specimens from 4 of 17 patients (23.5%). One of 17 patients had multiple recurrences with high grade dysplasia, which were positive for HPV HR types. Of 11 cases with dysplastic changes of mild to severe degree, 6 were positive for HPV HR types (54% of all dysplastic cases), while 5 were negative including one case of severe dysplasia (Table). HPV HR types were not seen in the 10 benign IP, which were without identifiable dysplastic changes.
Conclusions: HPV HR types are detected more often in dysplastic epithelium in sinonasal IP, which corresponds to morphologically neoplastic change. This test provides histological localization of HPV infection, which may be a valuable diagnostic marker for evaluating premalignant conditions. HPV HR types are also seen in a recurrent IP.

1514 Chronic Intermittent Hypoxia Regulates Hypoxia-Inducible Factor - 1alpha in Cancer Cells and Leads to an Aggressive Phenotype

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Background: Hypoxia is a common finding in solid tumors. It is a chronic intermittent state due to the abnormal tumor vasculature. Previous studies characterizing biological changes associated with hypoxia in cancer cells have been carried out under acute or transient hypoxic conditions, usually less than 24 hours. How cancer cells respond to a chronic intermittent hypoxic state is largely unknown.
Design: As a first step to evaluate this question, we studied in vitro cancer cells cultured under chronic intermittent hypoxic conditions. We alternated cycles of hypoxic (6

days) and normoxic (1 day) culture conditions for a period 1 to 2 months. We defined this regimen as hypoxia/reoxygenation cycles. For this study, SCC9 (head and neck squamous cell carcinoma) and LNCaP (prostate carcinoma) cell lines were used. We compared the levels of HIF-1alpha in cells exposed to 4 cycles of alternating hypoxia/normoxia and in cells grown for 4 weeks in normoxic conditions. In addition, these cells were also submitted to acute hypoxic conditions and HIF-1alpha levels were evaluated.
Results: The basal level of HIF-1alpha was increased after 4 cycles of hypoxia/reoxygenation as compared to cells grown in continuous normoxia. Most importantly when submitted to acute hypoxia, cells previously cultured under hypoxia/reoxygenation conditions had a significantly less degree of HIF-1alpha upregulation when compared to cells previously cultured in normoxic conditions. Hypoxia/reoxygenation cycles also increased PHD2 – an iron/oxygen-dependent hydroxylase which is involved in HIF-1alpha degradation under normoxic conditions. VEGF expression and cancer cells migration and invasion were also increased in cells post hypoxia/reoxygenation cycles. Finally, typical neuroendocrine differentiation was demonstrated in LNCaP cells after hypoxia/reoxygenation cycles and proliferation of SCC9 cells was increased as compared to that of LNCaP cells and thus is cell type dependent.
Conclusions: Cancer cells respond differently in conditions of chronic intermittent hypoxia or acute hypoxia. Chronic intermittent hypoxia tends to drive cancer cells toward a more aggressive phenotype. The study provides additional evidence that regulation of HIF-1alpha is complex and may be associated with epigenetic events. Detailed mechanistic study is warranted.

Pediatrics

1515 Expression of Survivin, Livin and Caspase 3 in Pediatric Malignant Perpheral Nerve Sheath Tumors (MPNST)

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Background: Survivin and livin are anti-apoptotic factors that block the transduction of apoptotic signals by inhibiting caspases. Increased levels of survivin in adult sarcomas are related with poor prognosis. Livin prognostic role in malignant neoplasms is controversial. We retrospectively investigated the expression of Survivin, Livin and Caspase 3 in a series of pediatric MPNST and their correlation with grading and prognosis.
Design: 21 MPNST enrolled in the Italian protocols RMS 88 and 96 were reviewed and FNCLCC grade was given. To determine Survivin and Livin expression, real-time quantitative RT-PCR was utilized on mRNA extracted from paraffin-embedded tissue sections, using GADPH as housekeeping gene. Immunohistochemical staining for caspase 3 was done in 13 case: the results were scored as 0 (< 5%), 1 (6-25%), 2 (26-50%), 3 (> 50%).
Results: Patients age range was 1-19 yrs; 9/18 suffered from neurofibromatosis 1 (NF1), in 3 the status was unknown. The mean follow-up was 4.8 yrs (range 1-19 yrs). Six died of disease, 2 for toxicity, 2 had relapses. In 4 cases either tumor grade or stage were not available. Survivin mRNA was detected in all MPNST, with high expression (ct ratio: 0.944922) in 10 and low in 11. High survivin expression was present in 2/5 G1, 2/3 G2 and 6/11 G3 MPNST with available grading. According to the stage (known in 18): 1/5 IRS I, 1/3 IRS II and 7/10 IRS III tumors had high survivin. NF1-associated and sporadic MPNST showed high survivin in 5/9 and 4/9 respectively. 1/3 with unknown NF1 status had high survivin. Prognosis was poor in 5/10 tumors with high survivin, and in 1/11 with low expression. 2 relapses occurred in cases with low survivin. Livin was detected in 4/18 cases, all but one in complete remission. Caspase 3 was positive in 2/13 (score 1, 3).
Conclusions: Survivin is expressed in all pediatric MPNST, without differences between NF1-associated and sporadic tumors. It is involved in the apoptosis inhibition, blocking Caspase 3, as confirmed by its negative staining in 76% of cases. High levels of Survivin are associated with advanced clinical stages (p=0.05) and aggressive clinical course. There is no relationship between tumor grade and survivin levels. Livin is poorly expressed and its role deserves further investigation.

1516 Morphoproteomic Profiling of a Recurrent Endodermal Sinus Tumor

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Background: Morphoproteomics is an important new tool for helping to guide targeted therapies for tumors which have failed to respond to conventional treatment or have no established protocol. It uses immunohistochemistry to quantify the expression of signal transduction pathways and effectors, to determine their compartmentalization, and for some, to determine their state of activation. This method has been successfully deployed on several types of neoplasms, but never previously on an endodermal sinus tumor (EST; yolk sac tumor).
Design: A pretreatment biopsy specimen taken from a now-recurrent, metastatic, and multidrug-resistant sacrococcygeal EST in a pediatric patient was received for consultative morphoproteomic analysis. The formalin-fixed, paraffin-embedded tissue was examined for expression and localization of four categories of proteins: 1) downstream pathways of convergence in signal transduction [Akt/mTOR, ras/Raf kinase/MAPK-ERK, and NF-κB]; 2) cell cycle impact [Ki-67, Skp-2, cyclin D1, and mitotic rate]; 3) antiapoptotic/tumorigenic/chemoresistance factors [bcl2, VEGF-A, GST-π, HIF-1α, and p-NF-κBp65], and 4) pro-apoptotic/antitumorigenic factors [ER-β and PPAR-γ]. The degree of expression in the nuclear, cytoplasmic, and plasmalemmal compartments was assessed and a tumor profile was constructed.
Results: The tumor has constitutive activation of all three signal transduction pathways of convergence examined, evidenced by phosphorylation and plasmalemmal translocation of mTOR and expression of its downstream effector, p-p70S6K, translocated to tumor cell nuclei; strong expression of p-ERK1/2 with nuclear translocation; and activation with nuclear translocation of p-NF-κB. Cell cycle analysis revealed a moderate mitotic rate (10 per 10 hpf) and a high percentage of cells entering S-phase, indicated by high percentages of nuclear Ki-67 and SKP-2. There was moderate expression of nuclear cyclin D1. Additionally, there was almost no expression of the anti-apoptotic protein, bcl-2, weak expression of GST-π, and low levels of HIF-1α and VEGF.
Conclusions: Morphoproteomic analysis provided considerations for combinatorial chemotherapy against this EST, beginning with oral rapamycin to block the mTOR pathway, followed by withdrawal for several days, with the intent of synchronizing entry into the S-phase of the cell cycle, at which point many should be susceptible to S-phase-active agents such as etoposide. This could be strategically combined with the NF-κB pathway inhibitor, bortezomib.

1517 Expression of Caspase 8, Bcl-2, Survivin and XIAP in Wilms' Tumors

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Background: Wilms' tumor is a common malignant tumor in children and is usually treated with various combinations of surgical resection, chemotherapy and radiotherapy. However, 15-20% of patients relapse with treatment and have low survival rates. Histologic evidence of anaplasia and high clinical stage are associated with bad prognosis. Apoptosis is an important process in the biologic behavior of tumors and may be activated by chemotherapeutic agents, as well as apoptosis inducing ligands, such as TRAIL. Drugs trigger apoptosis through the mitochondrial pathway which is inhibited by bcl-2, whereas TRAIL induces apoptosis through binding with its receptors and activation of caspase 8. Both pathways are inhibited by XIAP and survivin. Because the balance of pro-apoptotic and anti-apoptotic proteins may dictate tumor behavior and response to treatment, we studied the expression of caspase 8 (pro-apoptotic) and bcl-2, survivin and XIAP (anti-apoptotic) proteins in Wilms' tumor specimens.
Design: Thirty four Wilms' tumor samples (26 favorable histology, 6 with anaplasia) were studied for the expression of caspase 8, bcl2 and XIAP and 16 (14 favorable histology, 2 with anaplasia) for survivin. Overall 8 patients were stage 1, 2 stage 2, 12 stage 3, 4 stage 4 and 6 stage 5. Paraffin-embedded tissue sections were stained using regular immunohistochemical protocols. Immunostaining was evaluated by 3 pathologists. An immunohistochemistry score (IHS) was generated by multiplying the intensity of staining (scored as 0, 1+, 2+, 3+) with the percentage of positive cells (0, 1=<10%, 2=10-50%, 3=51-80%, 4=>80%). Low expression was defined as score 1-3, medium as 4-6 and high as 7-12.
Results: The majority of Wilms' tumors (68%) had low expression of caspase 8 (IHS <3), and moderate to high (IHS 4-12) expression of bcl-2 (65%), XIAP (91%) and survivin (81%). High bcl-2 expression (IHS >6) was found in tumors with anaplasia (5/6) and bilaterality (5/6).
Conclusions: Wilms' tumor, like neuroblastoma, often has no or low caspase 8 expression and therefore may not be amenable to treatment with TRAIL. High Bcl-2, XIAP and survivin expression suggests that they may serve as potential therapeutic targets.

1518 Expression of Insulin-Like Growth Factor Type 1 Receptor (IGF-1R) in Ewing Sarcoma Family Tumors (ESFT)

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Background: IGF-1R is a highly conserved transmembrane tyrosine kinase, which mediates mitogenic, differentiation and anti-apoptotic pathways. It is expressed at high levels in embryonic stem and cancer cells and at low levels in most adult differentiated tissues. Signaling pathways initiated by binding of IGF-1 to IGF-1R are of pivotal importance in human carcinogenesis. IGF-1R has been reported to be involved in the growth and survival of ESFT, and blockade of IGF-1R signaling in combination with chemotherapeutic agents induced significant ESFT cell growth in vitro. Because the IGF-1/IGF-1R pathway is involved in the growth and survivial of ESFT cells and circulating levels of IGF1 are usually increased in ESFT patients, we explored the expression of IGF-1R in tumor tissues form ESFT patients.
Design: We examined the expression of IFG-1R by immunohistochemistry in 28 cases from formalin-fixed paraffin-embedded tumor tissues, obtained from the files of Laboratory of Patology at NCI/NIH, and by immunoblotting in 15 ESFT cell lines (TC-32, TC-71, TC-248, TC-268, 5838, SK-N-MC, TC-300, TC-324, TC-389, TC-390, TC-392, TC-393, TC-394, TC-399, TC-400). A commercially available anti-IGF-1R polyclonal antibody was used.
Results: All ESFT cell lines expressed IGF-1R by Western blot, 5 of them expressed high levels, 6 expressed medium levels, and 4 low levels. By immunohistochemistry 72% of the cases expressed medium to high IGF-1R in tumoral cells, varying from 10-90%.
Conclusions: Our data demonstrated that IGF-1R is highly expressed in ESFT tissue and cell lines and therefore, may serve as a target for novel therapeutics interventions.

1519 Accelerated Villous Maturation Is Significantly Associated with Idiopathic Preterm Labor

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Background: Preterm birth is defined as a delivery before 37 weeks' gestation. Although it occurs in only 10% of pregnancies, complications account for the majority of neonatal morbidity and mortality. Amnionic infection is thought to be the leading cause of preterm labor (PTL) and preterm premature rupture of membranes (P-PROM), because cultures are positive in 61% of cases compared to 21% of controls. The remaining one-third