endophthalmitis was seen in children, due to Toxocara canis. The commonest cause of bacterial endophthalmitis was Staphylococcus aureus. PCR was used for the 20% of cases but with only 2 cases showing positive results. The majority of the patients with endopthalmitis (60%) underwent enucleation or an irreversible visual loss.
Conclusions: Fungal organisms are the commonest causes of endophthalmitis in our center. The yield of finding the organisms on vitreous cytologic preparations is 60% as compared to cultures (80%). PCR has a high diagnostic yield in viral and nematode endophthalmitis.

Pathobiology

1397 Angiogenic Histogenesis of Stromal Cells in Hemangioblastoma: Expression of Novel Endothelial Markers

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Background: Hemangioblastomas are uncommon central nervous system tumors which can occur sporadically or in von Hippel-Lindau (VHL) disease. Controversy regarding the origin of characteristic stromal cells is responsible for categorizing hemangioblastoma as "tumors of uncertain histogenesis" in the current WHO classification of brain tumors. Failure to specify the histologic origin of the stromal cells in hemangioblastoma has limited our understanding of its basic biology with possible treatment implications. The aim of this study was to investigate the expression of two novel endothelial markers (D2-40, a lymphatic marker); and CD105 (endoglin, a marker for neovascular proliferation) in hemangioblastoma, and to determine whether the expression of these immunohistochemical (IHC) markers can contribute to the understanding of the histogenesis of hemangioblastomas.
Design: A computer search of our hospital identified 27 cases of hemangioblastomas between 1997 and 2005, consisting of 10 spinal, 9 cerebellar and 8 cerebral hemangioblastoma. Four cases were associated with VHL disease. Immunostaining was performed on formalin-fixed, paraffin embedded sections using automatic immunostainer with appropriate positive and negative controls. Intensity was graded from 0-3 with a score 0 for no staining and 3 for maximal intensity. Cases which showed weak or <5% staining were considered negative.
Results: All cases of hemangioblastoma showed strong IHC staining for endoglin (CD105) in the vascular channels, but negative D2-40 staining. However; the stromal cells were positive in 14/27 (52%) for endoglin and 10/27 (37%) for D2-40. Seven of the D2-40 positive cases were negative for endoglin and six cases were negative for both endoglin and D2-40.
Conclusions: Although stromal cells of hemangioblastoma are classified as undifferentiated mesenchymal tumor, they demonstrate positivity for the novel endothelial markers D2-40 and endoglin, suggesting an endothelial histogenesis.

1398 Angiogenic Ability of Metastatic Squamous Carcinoma in the Cervical Lymph Nodes from an Unknown Primary

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Background: Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) molecules secreted by tumour cells and other surrounding benign cells are the principal inducers of neoangiogenesis in the growth of solid tumours beyond a size of 3-4mm. The isoforms of VEGF: $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ induce angiogenesis by endothelial cell proliferation and increasing vascular permeability. Metastases from unknown primary tumours (MUP) are not an uncommon clinical problem, especially in the head and neck. The phenomenon of a tumour that is clinically undetectable at the primary site yet capable of metastasis is intriguing and begs a biological explanation.
Design: The aim of the study was to study the angiogenic differences between metastasis of squamous carcinoma with unknown primary (MUP) and metastasis of squamous carcinoma with known primary (MKP) We have investigated the expression of the angiogenic molecules VEGF and bFGF in cervical lymph node (LN) metastasis of squamous carcinoma: 50 with unknown primaries (MUP) and 52 with known primaries (MKP) by immunohistochemistry performed on paraffin sections. We have also performed RT-PCR analysis of the expression of VEGF isoforms ($VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$) in 26 cases of MUP and 22 cases of MKP. RNA was extracted by guanidium-phenol-chloroform method and cDNA was prepared. Only those cases which were positive for housekeeping primers like underwent and RT-PCR analysis using the VEGF primers which gave a band size of 197bp with $VEGF_{121}$, 347bp with $VEGF_{165}$ and 419bp with $VEGF_{189}$.
Results: The immunohistochemical data showed that MKPs had significantly higher expression of VEGF (X^2 =46.2;p<0.001) and bFGF (X^2=22.5; p< 0.001) as compared to MUPs. The RT-PCR analysis showed that MKPs had increased expression of $VEGF_{121}$and $VEGF_{165}$ isoforms than MUPs.

VEGF isoform ($VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$) expression in lymph node metastasis with MUP and MKP

VEGF isoform	MUP (ng/µl) (n=22)	MKP (ng/µl) (n=26)	p value
$VEGF_{121}$	1.562±0.498	3.341±.906	0.002 (significant)
$VEGF_{165}$	1.058±.366	2.064±0.6	0.007(significant)
$VEGF_{189}$	0.684±.252	1.745±1.768	Not significant

MUP: Metastasis of unknown primary; MKP: Metastasis of known primary
Conclusions: Presuming, that tumour cells in the metastatic LNs reflect the primary tumour characteristics, we suggest that MUPs represent metastasis from tumours that have not established at the primary site due to their poor angiogenic phenotype.

1399 Differential Topographic Expression and Telomerase/Telomere Profiles Determine the Kinetic Advantage in Follicular Thyroid Carcinomas

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Background: The kinetic differences and telomerase/telomere profile by topographic compartments in follicular thyroid lesions have not been studied to date.
Design: We selected adenomatous hyperplastic nodules (FTHN, 18), adenomas (FTA, 19), carcinomas (FTC, 15 minimally-invasive and 15 widely-invasive), and anaplastic carcinomas (ATC, 10) (WHO criteria) to analyze by topographic compartments (internal/peripheral): Ki-67 and telomerase immunostaining, in situ end labeling (ISEL) of DNA fragments, telomere PNA-FISH and low-density selective cDNA array (LD-SelGEA; telomerase, p53, mdm2, p21, cdk2, cyclin E, pRB, Egr2, JunB, and FosB). Total RNA was extracted, cleaned from normal and neoplastic tissues (RNeasy columns), first-strand cDNA synthesized using T7-(dT24)-oligomer and used as template for cRNA synthesis. The cRNA was fragmented, Cy3-/Cy5-labeled, and hybridized to LD-SelGEA noncompetitively, cross-validating the results (expression factor>2, significance<0.01). Variables were studied regarding the histological diagnosis and molecular profile: RAS mutation (8 FTA, 7 minimally-invasive FTC, 5 widely-invasive FTC, 4 ATC), PAX8/PPARγ fusion gene (2 FTA, 1 minimally-invasive FTC, 7 widely-invasive FTC), and TP53 LOH/mutation (4 ATC) and combinations (3 widely-invasive FTC, 2 ATC).
Results: Internal compartments of benign lesions and peripheral compartments of malignant lesions revealed the most advantageous kinetic (increased Ki-67/ISEL index, due to significantly decreased ISEL index). Telomerase expression was significantly higher in internal compartments (p<0.001) and in malignant lesions (p<0.001), which only correlated with telomere PNA-FISH positive cells in internal compartments. Peripheral telomere PNA-FISH>20% was observed in high-grade lesions (widely-invasive FTC and ATC) only. Telomerase/telomere indices directly correlated with the kinetic index, being significantly higher in high-grade malignancies with multiple genetic alterations (PAX8/PPARγ-RAS in widely-invasive FTC and RAS-TP53 in ATC) and cases with upregulation of p53, cyclin E, Egr2, JunB and FosB at LD-SelGEA.
Conclusions: The kinetic advantage predominates in internal compartments of benign lesions and in peripheral ones of malignant lesions, due to inverse and opposite proliferation/apoptosis correlations. This kinetic profile directly correlates with the telomere-telomerase index, especially in FTC with multiple genetic abnormalities.

1400 Up Regulation of P-Cadherin in Uterine Fibroids Shows a Race Specific Difference

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Background: Multiple larger uterine fibroids are a cause of pelvic discomfort in women. While their pathogenetic basis is not well defined, African American women (AAW) are more likely to have this type of fibroid than Caucasian women (CW). P-cadherin (P-cad), N-cadherin (N-cad) and beta-catenin (B-cat) expression in fibroids have not been well studied, especially for race specific differences.
Design: 28 sequential AAW fibroid uteri were identified (Age: 41 ± 5 years; Myoma size: 0.2 – 11 cm; Endometrium: 13 proliferative, 9 secretory and 6 inactive). An age, menstrual cycle and fibroid size-matched CW group was selected for comparison. These specimens provided 51 AAW and 36 CW fibroids for analysis. Routine DAB immunostaining for P-cad, N-cad and B-cat was performed on formalin-fixed paraffin-embedded tissue, according to manufacturers' recommendations. Each marker's expression was blindly evaluated in the myometrium and fibroids, using the following intensity scoring system: undetectable (0+), weak (1+), moderate (2+) and strong (3+). Differential expression between the fibroids and matched myometrium was calculated. Statistical analysis was performed.
Results: All specimens together: P-cad expression was higher in fibroids (2.0+ ± 0.8+) than myometrium (1.2+ ± 0.7+; p<0.001). N-cad expression was higher in fibroids (1.8+ ± 0.7+) than myometrium (1.0+ ± 0.6+; p<0.001). B-cat expression was higher in fibroids (0.5+ ± 0.5+) than myometrium (0.2+ ± 0.4+; p=0.005). Expression by race and tissue type is shown in the Table.

Mean Expression Scores By Race, Tissue Type And Differential Expression

	African American Women		Caucasian Women		Matched Expression Differences	
	Fibroid	Myometrium	Fibroid	Myometrium	AAW	CW
P-cad	1.9+ ± 0.8+	1.3+ ± 0.6+	2.1+ ± 0.8+	1.1+ ± 0.7+	0.5+ ± 0.9+	1.1+ ± 1.1+
N-cad	1.7+ ± 0.7+	1.0+ ± 0.7+	1.9+ ± 0.7+	1.1+ ± 0.6+	0.7+ ± 0.8+	0.8+ ± 0.8+
B-cat	0.5+ ± 0.5+	0.1+ ± 0.3+	0.6+ ± 0.5+	0.2+ ± 0.4+	0.4+ ± 0.6+	0.4+ ± 0.5+

Conclusions: Uterine fibroids have significantly increased P-cad, N-cad and B-cat expression when compared to the myometrium. While no race difference was identified for N-cad (p=0.358) or B-cat (p=0.954), P-cad showed significantly greater expression in CW than AAW fibroids when compared to the matching myometrium (p=0.007). Thus, it could be hypothesized that differential up-regulation of P-cad in fibroids may contribute to the biologic differences between African American and Caucasian women.

1401 Automation of TMA Interpretation through a Combination of Epithelial-Recognition and Specific-Recognition Algorithms

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Background: Tissue microarrays (TMAs) are a form of high throughput screening akin to cDNA microarray and proteomic analyses, yet, unlike the latter types, are based on manual construction and subjective interpretation. Because of the increasing demand for TMAs predicted to occur over the next decade, we felt it necessary to investigate whether their interpretation could be completely automated.

Design: In this study we used TMAs made from breast, colon and lung cancer and analyzed each TMA for two nuclear, cytoplasmic and membrane immunocytochemical markers that were either homogeneously or heterogeneously expressed and compared the algorithmic measurements with the subjective ones of 3 pathologists. For the breast carcinoma TMA, the targets were: nuclear (ER, p53); cytoplasmic (PDGFRα, COX-2); membrane (Her-2 / neu, EGFR). For the colon carcinoma, the targets were: nuclear (Ki-67, p53); cytoplasmic (COX-2, CEA); membrane (Her-2 / neu, EGFR). For the lung carcinoma, the targets were: nuclear (Ki-67, TTF-1); cytoplasmic (CEA, cytokeratin 7); membrane (Her-2 / neu, EGFR).
Results: We report the successful creation of both epithelial recognition algorithms (ERAs) based on selective imaging properties (Gaussian kernel and elongation ratio) and specific recognition algorithms (SRAs) based on pixel colors (RGB) and gray scale intensities that can analyze virtual slides created by TMA scanning. These algorithms rapidly identify cancer cells in a background of stroma, filter out this non-cancer background, successfully compartmentalize the cancer cells into nucleus, cytoplasm and membrane and then accurately quantitate the degree of immunocytochemical staining. The algorithm-based measurements strongly correlate with the subjective measurements with the strength of correlation being greater for nuclear (0.9) than membrane (0.8) than cytoplasm (0.7) (Confidence Intervals: 0.59, 0.95). The algorithmic measurements exhibit no interobserver, intraobserver or fatigue variability which is observed for the subjective interpretations.
Conclusions: These digital algorithms can presently be used to interpret virtual slides of TMAs in a more objective, more quantitative and more reproducible manner. Because of the increasing use of TMAs in both biomarker discovery and validation predicted to occur in the near future, this automatic interpretation is a prerequisite for making TMAs truly high throughput.

1402 hnRNP M Protein Expression in Cancer and Nornal Tissues

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Background: Carcinoembryonic antigen (CEA) is involved in the metastatic spread of colorectal cancer by protecting cancer cells from undergoing apoptosis. The CEA receptor (CEAR), also known as hnRNP M4 protein, has been identified on the surface of cancer cells and macrophages. Previous studies demonstrated that the full length isoform of hnRNP M4 has nuclear localization in the MIP 101 colorectal cell line.
Design: We compared expression of hnRNP protein in matched pairs of cancer and normal tissues. We also assessed hnRNP distribution and mRNA splicing in several types of adenocarcinoma of known stage and grade. We have made specific antibodies against hnRNP isoforms; these antibodies were used to stain siRNA treated and control CEA producing cells.
Results: High levels of hnRNP were detected in normal colon, endometrial and breast tissue. The expression of hnRNP was suppressed in colorectal carcinoma and endometrial carcinoma versus matched adjacent normal tissues. In contrast, the hnRNP level is higher in breast carcinoma versus control. This distribution of hnRNP protein in CEA-producing cells changes its nuclear functions, including transcription and splicing of target genes, which impacts the resistantance of these cells to apoptosis and metastatic spread.
Conclusions: Carcinomas differ in their ability to form metastasis. Our analysis revealed changes in the hnRNP protein expression in various cancer and normal tissues. We conclude that, as a CEA-interacting protein, hnRNP correlates with metastatic potential. Redistribution of hnRNP between cellular compartments plays a crucial role in the CEA-related proliferative and anti-apoptotic function of cancer cells.

1403 Histone H1 Phosphorylation as a Marker of Premalignancy

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Background: The linker histone H1 is involved in maintaining higher-order chromatin structure. H1 is phosphorylated by cyclinE/CDK2 during the G1/S-phase transition, resulting in a more open chromatin conformation. We hypothesize that the consequences of unregulated H1 phosphorylation, which can occur with increased cyclinE/CDK2 activity, in vivo may be a higher susceptibility to cellular transformation.
Design: Paraffin sections of mammary glands and prostate from BrdU-injected wild-type and tumor-forming transgenic mice were stained by co-IF using a polyclonal P-H1 antibody (Upstate) and a monoclonal BrdU antibody conjugated with FITC (Becton-Dickenson). Nuclei were counterstained with DAPI. Mammary glands and prostates were analyzed from early time-points where glands appear histologically normal. Ten fields from three separate glands (n=30) were captured with a fluorescent microscope, and only epithelial cells were analyzed for the presence of P-H1 or BrdU. In addition we analyzed by immunohistochemistry, P-H1 levels in 175 human breast cancer and adjacent normal epithelium samples, and correlated the levels of P-H1 with known prognostic factors, including proliferative markers.
Results:

Mouse mammary gland data

	WT (6,8,12 wks)	C3 (6,8,12 wks)	WT (12 wks)	p53 KO (12wks)
P-H1	2%	16-20%	5%	25%
BrdU	<2%	10%	3%	11%

Mouse Prostate Data

	WT (6 wks)	TRAMP (6 wks)
P-H1	1%	12%
BrdU	1%	8%

In human cancerous tissue, P-H1-positive cells ranged from 2 to 22%. P-H1 expression was always higher in cancer than in adjacent normal epithelium. In addition, P-H1 was strongly correlated with Ki-67 index (cc:0.455, p=0.0001), high SPF, and histological grade.
Conclusions: Our data support the hypothesis that in breast and prostate cancer, one of the consequences of high cyclin E/CDK2 activity is aberrant H1 phosphorylation, which may render cells more susceptible to transformation, and thus, results in a mutator phenotype. High levels of P-H1 observed in histologically normal-appearing epithelium of premalignant tissue suggest that P-H1 may be a marker of early tumorigenesis. Based on the initial staining in human breast cancer, we are correlating levels of P-H1 with patient outcome and response to treatment.

1404 Perineural Invasion In Vitro Model Derived Supernatant Confers Neurogenic Potential

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Background: Our previous study indicated that prostate cancer cells and nerves can establish symbiosis and gain mutual growth advantages. This study will demonstrate the effects of perineural invasion (PNI) in vitro model-derived supernatant on neurite outgrowth of neuroblastoma cells *N1E-115*.
Design: Mouse neuroblastoma *N1E-115* cells were induced by serum starvation for 24 hours prior to the experiment. Induced N1E-115 cells were seeded at equivalent densities in 6-well plates containing 50% fresh DMEM with 5%Nu-serum. Supernatant from the DU-145/DRG neuroepithelial co-cultures (PNI) in vitro model and DU-145 cells alone or DRG alone controls (50% volume) were subsequently added. Plates were incubated at 37°C and 10% CO_2 for 48 hours. Then each sample was photographed at 100X at locations of high neurite density and high cell density location. Neurite length was computed via Optimas 6 image analysis suite; total number of neurites and the number of cells with neurite sprouting were counted manually.
Results: Cells grown in DU-145/DRG perineural invasion co-culture supernatant had significant increases in *total length of neurite outgrowth* (41.585 vs. 15.3, P=0.0037) and *neurite sprouting number* (11.5 vs. 4.33, P=0.0166), compared to cells grown in the supernatant of ganglion alone group. However, the *percentage of cells with neurites* cultured in the co-culture model supernatant was not higher than controls.
Conclusions: This study suggests that micro-environmental factors derived from PNI in vitro model enhance not only cancer cell growth and survival, as we previously published, but also neurite growth. Understanding the molecular mechanism of this carcinoma/nerve interaction would have therapeutic implications.

1405 PTOV-1 Overexpression in Human Epithelial Tumors: A New Marker Associated with Neuroendocrine and High Grade Malignant Tumors

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Background: PTOV1 is a novel protein encoded by 12-exon gene localized in chromosome 19q.13.3. recently identified as androgen induced gene involved in prostate cell proliferation and in prostate human cancer. Preliminary data indicates that PTOV-1 can be related to flotilin ,integrins and other cellular factors involved in cancer progression. Because PTOV1 has not yet studied in non-prostate tumors, we underwent an immunohistochemical study in a large series of human tumors in order to describe the expression of this novel oncogenic factor.
Design: 183 human tumors of low /high grade from the files of Pathology Department were selected: 22 lung squamous (LSC), 22 breast ductal (BDC)and lobulillar (BLC), 10 endometrial (EC), 10 ductal pancreatic (DPA) ,10 hepatocarcinomas (HCC), 11 squamous skin carcinomasSSC), 19 malignant melanomas (MM),20 mucinous (MOC)and serous (SOC) ovary carcinomas, 10 gastric (GC) and 10 colonic adenocarcinomas (CA);12 cerebral gliomas (CG),12 clear cell renal (CCRC) ,11 bladder carcinomas (UBT) and13 neuroendocrine tumors (NET) .TMAs of different and representative tumors as well as normal control tissues were constructed and immunohistochemical assay with PTOV-1 (polyclonal 1:40 dilution),Ki67(Dako)and Chromogranin A (Novocastra) with Envision method was performed. Intensity and percentage of immunostain was Hscored (scale 1 to 300) and considered overexpression > 100.
Results: PTOV1 overexpression was observed in the most of human epithelial tumors compared with normal tissues. PTOV-1 highlight neuroendocrine cells as strongly as chromogranin. In >90% NET showed intense immunoreactivity. In the other set of tumors the percentage of positive cases were: 85% MM; 100% UBC; 92% CCRC; 90% EC; 90% CA,70% intestinal-type GC; 80% DBC, 70% LBC; 87.5% MOC , 37.5% SOC; 75% HCC, 70% DPC; 73% SLC and 60% SSC. High grade tumors showed higher nuclear PTOV1 than low grade tumors.
Conclusions: PTOV-1, a protein initially associated with prostate tumors is strongly expressed in neuroendocrine cells and in neuroendocrine tumors. Moreover, mild PTOV1 overexpression was detected in a wide series of different malignant tumors emphasizing the immunoreactivty of high grade carcinomas. This preliminary study points out that PTOV-1 may play an important role in the biology and development of neuroendocrine tumors. Ongoing studies are carried to understand the association of PTOV-1 with high grade carcinomas.

1406 Comparative Molecular Pathology of Adenoid Cystic Carcinoma from Different Sites: A Microarray-Based CGH Analysis

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Background: Adenoid cystic carcinoma (ACC) is a tumour that can arise at multiple sites in the body. Despite similar morphological features, biological behaviour can significantly differ between primary sites: salivary ACC can be an aggressive disease, whilst breast ACC has a uniformly good prognosis. This study aims to investigate molecular alterations within ACC using microarray CGH and compare these depending on site of origin.

Design: 8 salivary ACC, 15 respiratory ACC, 6 breast ACC and 7 salivary ACC metastatic to lung were retrieved. Tumour cells were microdissected from paraffin embedded sections to ensure >75% neoplastic cells. Micro-array CGH analysis was carried out on non amplified DNA, using a 1Mb resolution platform. Genomic changes significantly more frequent in tumours from each site were identified by an adjusted multi-Fisher's exact test.
Results: Successful hybridisation was achieved in 29 cases. Recurrent changes were seen at all sites and included deletions of 6q24.1-q24.3 (48% of cases), 12q13.11 (65%) and 13q21.31 (52%), and gains of 2p21 (42%), 4p14 (65%), 9q34.2 (65%) and 11p15.5 (72%) In addition, breast ACC showed significant differences compared to other sites, including loss of 6q24.1 (p=0.0006) and 6q25.1-q27 (p=0.027). Metastatic ACC were more likely to show extensive and complex losses of chromosome 12 compared to other sites: 12p12.3-p12.1, (p=0.024), 12q13.12-13.13 (p=0.036), 12q13.2, 12q14.1 and 12q15-q21.31 (all p=0.007).
Conclusions: These results show that ACC arising at different sites share similar alterations and are likely to evolve through similar molecular genetic pathways. Several candidate cancer genes map to the regions identified, including tumour suppressor genes mapping to regions previously highlighted by LOH studies, corroborating target regions of interest to investigate in these tumours. This study also demonstrates significant differences between breast ACC and other primary sites which may relate to their favourable biological behaviour. Metastatic ACC displayed more complex molecular genetic profiles than all primary ACC, which would be consistent with more aggressive behaviour.

1407 Seminal Fluid as a Local Source of IGF-I in the Prostate: Implications for Prostate Cancer

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Background: In human populations, epidemiological studies have suggested that elevated circulating levels of IGF-I may be associated with an increased risk for prostate cancer. However, there have been conflicting reports regarding this correlation. Among the possible reasons for these conflicting reports is that the serum levels of IGF-I do not accurately reflect the local concentrations of IGF-I in the prostate. Support for this concept comes from studies examining the effect of a liver specific disruption of the Igf1 gene. Mice homozygous for this disruption show a dramatic reduction in serum IGF-I levels but display normal development and achieve normal size. These results indicate that tissue production of IGF-I plays a greater role in tissue homeostasis than previously realized. We have identified a potential source of local IGF-I in the peripheral zone of the prostate. The ejaculatory ducts open into the urethra upstream of the peripheral zone terminal ducts and we find that seminal vesicle fluid (SVF) contains relatively high levels of IGF-I.
Design: We have examined the potential local consequences of SVF derived IGF-I in the prostate, identifying the activated IGF-I receptor in regions of intermediate basal cell (IBC) hyperplasia which occur primarily in the peripheral zone of the prostate downstream of the ejaculatory ducts. We also examined cell proliferation and epithelial barrier function in response to SVF.
Results: We find that the IGF-I receptor is expressed and activated in p63 positive cells within areas of IBC hyperplasia. In vitro we find that SVF and promotes the proliferation of both normal and malignant prostate cells and this stimulation is more potent than equivalent concentrations of fetal bovine serum. We also find that addition of SVF to CD133 positive prostate cancer prostate tumor initiating cells drives proliferation in these cells. Surprisingly, SVF had only slight effect on epithelial barrier function as measured by electrical flux.
Conclusions: These results suggest that IGF-I contained within the seminal vesicle fluid serves as an initiating factor in the development of prostate cancer. However, SVF does not significantly alter epithelial barrier function suggesting that local injury might be required for infiltration of SVF into the basal cell layer.

1408 Overexpression of PDEF in the Progression of Breast and Prostate Carcinomas

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Background: PDEF (Prostate Derived Ets Factor) belongs to the Ets family of transcription factors that play an important role in normal as well as neoplastic development. There are scant and conflicting data on PDEF expression in breast and prostate tumors.
Design: A rabbit polyclonal antibody to PDEF was prepared and reacted with tissue microarrays (TMAs) consisting of 1 mm cores of 62 benign breast tissues, 46 *in situ* carcinomas, 65 invasive ductal carcinomas and 39 invasive lobular carcinomas. Further, the antibody was reacted with TMAs from 290 benign prostate tissues, 109 PIN samples and 230 prostate carcinomas. The average nuclear staining intensity and the percentage of stained epithelial cells were evaluated. Thresholds were set to separate normal from elevated expression levels.
Results: Relative overexpression of PDEF was identified in 11 of 62 (18%) benign breast tissues, 23 of 46 (50%) DCIS lesions, 30 of 65 (46%) invasive ductal carcinomas, and 20 of 39 (51%) invasive lobular carcinomas. Further, of the 9 matched samples of benign breast and tumor tissues from same patients, 8 showed an increase in the number and/or intensity of PDEF expressing epithelial cells in tumors. Relative overexpression of PDEF was also identified in 79 of 290 (27%) benign prostate tissues, 36 of 109 (33%) PIN samples, and 92 of 230 (40%) prostate carcinomas. Importantly, comparison of the matching samples of cancer *versus* benign prostate tissue and cancer *versus* PIN showed that in 68% and 70% cases respectively, increased expression of PDEF was seen in the invasive carcinoma cells.
Conclusions: These results, together with restricted expression of PDEF in normal human tissues and its pro-migratory and pro-invasive function in epithelial cell biology, strongly implicate PDEF in the progression of both breast and prostate carcinomas and suggest that this biomarker may be a novel therapeutic target in these tumors.

1409 GM-CSF Promotes Breast Cancer Bone Metastasis

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Background: Advanced breast cancers most frequently metastasize to bone and develop osteolytic lesions for which underlying mechanisms are poorly understood. Growing evidence suggests that a variety of growth factors may be associated with breast cancer bone metastasis. The objective of this study is to investigate whether or not granulocyte macrophage-colony stimulating factor (GM-CSF) plays a role in breast cancer bone metastasis.
Design: Thirty-five cases of bone metastasis from primary breast invasive ductal carcinoma from Henry Ford Hospital from year 1993-2006 were studied; all cases had biopsy-approved bone metastasis. Formalin-fixed paraffin-embedded tissue sections from bone metastases were deparaffinized and antigen retrieval was performed. Serial tissue sections were pre-incubated with peroxide block solution and then incubated with polyclonal antibodies against human GM-CSF. Immunostaining was detected with AEC+ chromogen using the Dako EnVision System. In a mouse model, briefly, a breast cancer cell line (MDA-MB-231) with predilection for bone metastasis was infected with retroviruses expressing GM-CSF small interference RNA (siRNA) and control luciferase siRNA and then stably selected with antibiotics. The knock-down of GM-CSF in cells was confirmed by Western blot analysis. Cells expressing GM-CSF siRNA or control cells were injected into mouse left ventricle. Three weeks after injection, X-ray was taken and histology was performed to determine bone metastasis.
Results: Thirty-two of 35 human breast cancer bone metastasis samples contained sufficient tumor cells for analysis. Twenty-four of 32 (75%) samples showed strong GM-CSF expression of metastatic breast tumor cells. In the animal model, we found that GM-CSF was also highly expressed in breast cancer cell line (MDA-MB-231). The depletion of GM-CSF using siRNA suppressed the osteolytic bone metastasis of MDA-MB-231 cells as well as the osteoclastic activities induced by GM-CSF in the mouse model of bone metastasis.
Conclusions: In summary, our results suggest that the up-regulation of GM-CSF in breast cancer cells may play an important role in breast cancer bone metastasis and progression.

1410 Expression of Neurotensin Receptor-1 (NTR-1) mRNA in Intestinal Laminal Propria Mononuclear Cells

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Background: Neurotensin (NT), a tridecapeptide secreted by specified cells (N cells) and some neurons, exists predominantly in small intestine and participates in the regulation of bowel function. It was also found to regulate lymphoid cells in their proliferation, traffic, adherence and chemotaxis capacity, in in-vitro experiments using lymphocyte preparations isolated from lymphoid tissue or blood. However, the existence of NT receptor on the intestinal laminal propria lymphocytes has not been shown in situ in human bowel. Here we demonstrate the first time in situ the strong expression of type-1 NT receptor (NTR-1) on colonic laminal propria mononuclear cells, predominantly lymphocytes.
Design: NTR-1 mRNA expression was determined by *in-situ* hybridization on formalin-fixed and paraffin-embedded human colon tissue. Tissue blocks of 21 cases of colorectal adenocarcinoma (male 13, female 8, mean age 63.4 yo), encompassing normal mucosa and adenomas, were used. A 322-bp fragment of hNTR-1 cDNA was subcloned into pBluescript II KS+ vector. Digoxigenin-labeled anti-sense and sense riboprobes were generated by *in-vitro* transcription. Anti-DIG-AP conjugate and BCIP/NBT were used in chromogenic detection for hybridization signals. The expression intensity was classified as negative, weak, moderate, and strong.
Results: Strong expression of NTR-1 was seen in the cytoplasm of more than 80% of the laminal propria mononuclear cells which are predominantly lymphocytes. The same pattern was seen in 100% of the colonic tissue specimens with similar intensity of expression, irrespective of the conditions of the epithelium (normal epithelium vs adenoma vs adenocarcinoma). The tumor-infiltrating lymphocytes, seen in some cases (5/21), showed no significant enhancement or attenuation of NTR-1 expression. NTR-1 expression in colonic crypts and superficial epithelial cells, however, varies significantly, which is none or weak in normal mucosa but progressively enhanced in adenomas and carcinomas. No association exists between the laminal propria mononuclear cells and the overlying epithelial cells in this regard.
Conclusions: This study illustrated a strong expression of NTR-1 in colonic laminal propria mononuclear cells, providing further evidence for a link between NT and the immune system, i.e. neuro-immune interactions. NT may play a role in regulation of enteric mucosal immune response.

1411 Prognostic Analysis of HER Pathway Expression by Objective Quantitative Analysis (AQUA™) in Two Cohorts Representing over 550 Non-Small Cell Lung Cancer Cases

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Background: Lung cancer is the leading cause of cancer deaths in the developed world thus, there is a critical need for better treatment options, as well as better prognostic and classifying biomarkers. The HER family is critically involved in the progression of non-small cell lung cancer (NSCLC). However, there is conflicting data concerning the prognostic value of HER family expression, specifically EGFR and Her2.

Design: In order to better understand the prognostic value of HER family members in NSCLC, automated quantitative analysis (AQUA™), a new method for performing highly reproducible and quantitative *in situ* protein expression analysis, was performed for each HER molecule on two individual cohorts of NSCLC, using the first cohort as a training set and the second as a validation set. The training set is comprised of 213 NSCLC tumors (collected at Yale University; New Haven, CT) and the validation set is comprised of 350 NSCLC tumors (collected at The Methodist Hospital; Houston, TX). For survival analysis of each marker, optimal cut points were generated using X-tile on the training set and subsequently applied to the validation set.
Results: Survival analysis of EGFR and Her2 on the training cohort revealed a significant correlation between elevated expression and decreased 5-year disease-specific survival [EGFR expression (top 37%): decrease in survival from 82 to 64%, Monte Carlo $p = 0.02$; HER2 expression (top 17%): decrease in survival from 78 to 57%, Monte Carlo $p < 0.0001$]. Application of the optimal cutpoint for EGFR to the validation cohort was not significant ($p = 0.96$) while application of the optimal cutpoint for Her2 showed significance ($p = 0.021$). Treating the validation cohort separately, there was no cutpoint at which increased EGFR expression affected decreased survival. Her3 and Her4 expression did not have a significant effect on survival in either cohort.
Conclusions: Given the robust and reproducible nature of AQUA, we can conclude from these studies that EGFR expression can influence survival in a subset of the population, but it is not necessarily predictive of outcome in the population as a whole. In contrast, we observed elevated Her2 expression to have a significant and consistent effect on overall survival in two discreet populations of NSCLC patients. Taken together, these data suggest that Her2, but not EGFR (nor Her3 and Her4), to be of significant prognostic value in NSCLC.

1412 AQUA™ Based Analysis of Thymidylate Synthase (TS) within Subcellular Compartments Reveals a Novel Biomarker for Prediction of Survival in Colorectal Carcinomas

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Background: Thymidylate synthase (TS) is critically involved in DNA synthesis. High expression levels have been shown to be a marker for decreased survival and response to therapy, such as 5-FU, a longstanding chemotherapeutic agent for colon cancer. Recently, it has been demonstrated that TS may have other cellular functions, including translational regulation. Thus the subcellular localization of expression may be an important predictive determinant. Here, we use AQUA, a new method for in situ determination of protein concentrations within subcellular compartments, to assess the prognostic and predictive value of TS expression as a function of subcellular localization.
Design: We examined a large cohort [n=518] of primary colon tumors obtained between 1970 and 1981, retrospectively collected from the Yale Pathology archives. We used X-tile for selection of optimal cut-point for continuous data on a test set representing one-third of the cohort, then applied this cut-point on a validation set representing the remaining two-thirds. Using quantitative AQUA data, we generated a ratio of nuclear to cytoplasmic expression to normalize for individual variation and artifacts.
Results: A high nuclear:cytoplasmic ratio (top 10%) resulted in decreased 5-year disease-specific survival by Kaplan-Meier analysis [65 to 45%; validation set p=0.010)] and by Cox univariate analysis [RR=1.61 (95%CI: 1.09-2.37; p=0.012)]. In order to assess the ability of this ratio to predict response to 5-FU treatment, we examined a subset of this cohort that was treated with 5-FU (n=80). Comparing this subset with the rest of the cohort and controlling for stage, age, gender and race, we observed that patients within the treatment group having a high nuclear:cytoplasmic ratio had a significant 2.9-fold greater risk of death (95CI: 1.12–7.56; p=0.029) as compared to patients with a low nuclear:cytoplasmic ratio. In contrast, patients with a high nuclear:cytoplasmic ratio within the non-treatment group had a non-significant 1.4 fold greater risk of death than patients with a low ratio (95CI: 0.85–2.35; p=0.19).
Conclusions: Taken together, a ratio of nuclear to cytoplasmic expression of TS appears to be a novel biomarker for predicting survival, and for predicting response to 5-FU treatment. Furthermore, these data demonstrate that subcellular localization of TS is critical in prediction of outcome in colon cancer.

1413 Urothelial Differentiation in Urothelial Carcinoma: A Bladder Cancer Stem Cell Model

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Background: As few as 2-5% of cells in solid tumors can grow indefinitely and colonize metastatic sites. Such "cancer stem cells" have been partially characterized in tumors from the brain and breast, but little is known about other sites, and no paradigm has been established to identify and isolate carcinoma stem cells. In most epithelia, the stem cell compartment abuts a basement membrane and is therefore called a "basal" cell, with more differentiated cell types populating positions closer to the surface. Here we show that urothelial carcinomas comprise distinct cell populations corresponding to basal (cytokeratin 17+/cytokeratin 20-), intermediate (ck17-/ck20-), and superficial (ck17-/ck20+) cells in normal urothelium. We further demonstrate enhanced tumor-forming potential of a subpopulation of basal-like cancer cells.
Design: We inoculated human urothelial carcinoma cell lines into immunodeficient mice and analyzed the resulting subcutaneous tumors by histomorphology, immunohistochemistry, and flow cytometry. We used fluorescence-activated cell sorting to isolate basal-like cells from digested tumors and tested their tumor forming potential *in vivo*.
Results: In human urothelial carcinoma xenografts from several different patients, we noted that tumor cells were organized in layers, with ck17 expressing basal-like cells ringing the basal aspect of tumor nodules, adjacent to tumor stroma, larger ck20 positive cells in the center of the nodule, and ck17-/ck20 cells intercalated in between. In one such xenograft, we found coexpression of ck17 in basal cells with a surface receptor predicted to bind to laminin in the basement membrane. We used antibodies against this receptor to isolate tumor cells from dissociated urothelial carcinoma xenografts with high or low expression levels. We found equal cell viability for the two populations *in vitro*. However, receptor-bright cells were at least 6-fold more potent in forming tumors *in vivo* than receptor dim cells and gave rise to tumors that were almost 5 times larger. Tumors from receptor-bright cells also recapitulated the full range of cell types in the original tumor.
Conclusions: These results illustrate a hierarchy of differentiation among carcinoma cells that corresponds to different abilities to form tumors. Our findings further implicate the basement membrane as a "niche" for carcinoma stem cells. Future studies will pursue the implications of these findings for refining cancer classification, prognosis, and therapy.

1414 Regulation of Cancer Stemness: Understanding Self-Renewal and Early Differentiation in Cancer Stem Cells

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Background: Similarities between stem cells and cancer cells led to the concept of the existence of a cancer stem cell. This concept was initially documented in haematological malignancies and has since been recognised in many tumours. The defining features of stem cells are their ability to self-renew and to generate differentiated progeny, properties shared by cancer stem cells. Teratocarcinomas are malignant tumours that occur in the gonads and are largely composed of undifferentiated cells with pluripotent capabilities. These tumours are often referred to as the classical stem cell tumour. The aim of our study was to establish gene expression profiles of self renewal and differentiation in normal stem cells as well as cancer stem cells using teratoma tumourigenesis as our model system thus allowing us to study genes that are required to drive cancer cell proliferation and progression.
Design: To do this, we performed expression array analysis on murine embryonic stem cells (ES) in their undifferentiated and differentiated states and compared them to murine pluripotent and nullipotent teratocarcinoma cells in both their undifferentiated and differentiated states, which represent our malignant/cancer stem cell. All microarrays were performed in triplicate using Applied Biosystems technology. Data was analysed using a combination of R and Spotfire® software programs. ES array data was compared to that of the malignant cell lines to obtain a list of differentially expressed genes in the non-malignant and malignant states. Differentiated and undifferentiated array data was compared for each cell line to generate a list of differentially expressed genes responsible for differentiation in both states. The gene ontology site, PANTHER, was used to identify the pathways, biological processes and molecular functions associated with significant genes.
Results: Putative lists of genes including homeobox transcription factors and hedgehog signalling pathway molecules have been compiled for those genes involved in self-renewal and early differentiation of normal and cancer stem cells. Validation of array data was performed on 47 gene targets using TaqMan® chemistry from Applied Biosystems.
Conclusions: We propose that these lists represent transcriptome profiles of self-renewal and differentiation in cancer stem cells and outline some of the pathways important in early differentiation in both the normal and malignant states.

1415 A Cancer-Specific Isoform of PCNA Is Linked to Breast Cancer: Implications for a Novel Cancer Biomarker

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Background: Our 2D-PAGE analysis of the breast cell DNA synthesome identified a unique acidic isoform of Proliferating Cell Nuclear Antigen (caPCNA) that is selectively expressed in breast cancer cells, but not non-malignant breast cells. Commercial antibodies to PCNA (e.g., PC-10ab) interact with both isoforms, and do not differentiate between PCNA isoforms in malignant and non-malignant breast cells. Structural analysis of the caPCNA protein led us to develop an antibody (caPCNAab) that selectively detects only the caPCNA isoform, and appears to selectively detect breast cancer cells.
Design: Immunofluorescence (IF) analysis was performed with caPCNAab and PC-10ab on normal human mammary epithelial cells (HMEC) grown in culture, non-malignant spontaneously immortalized HMECs and both tranformed HMEC's and MCF-7 cells. Paraffin sections from 20 cases of breast cancer (ductal carcinoma in-situ, invasive and metastatic disease) and 10 cases (normal breast tissue and benign breast disease) were selected for immunohistochemical study with caPCNAab and PC-10ab, along with a breast tumor progression series tissue array.
Results: Immunofluorescence analysis showed caPCNA antibody specifically recognizes the transformed HMEC and MCF-7 cancer cells, and did not stain normal primary HMEC cells or immortalized HMEC cells. Immunohistochemistry data showed that caPCNA positively stained only breast cancer cells, and not normal breast tissue and most atypia. There was a correlation between the intensity of staining and tumor grade.
Conclusions: We report here the development of a new antibody that specifically detects the caPCNA isoform uniquely expressed in breast cancer cells. The caPCNA isoform may be a *bona fide* marker of breast cancer, and may be useful for identifying true atypia from very early stage DCIS and LCIS.

1416 Functional Analysis of an Xq26 Micro RNA Polycistron Gene That Collaborates with p27kip1 Loss in Tumorigenesis

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Background: Loss of p27kip1 is a common finding in many human malignancies and is associated with worse prognosis in carcinomas arising in the breast, lung, and colon as well as in lymphoma. We performed a retroviral insertional mutagenesis screen in p27-null mice to identify oncogenes that collaborate with p27-loss in tumorigenesis. Mapping of proviral common integration sites identified two genomic loci that collaborate with p27-loss: Myc and a recently annotated 0.9 kB micro RNA cluster on Xq26 containing 6 micro RNAs (miR-18b, miR-19b, miR-20b, miR-92-2, miR-106a, and miR-363). RT-PCR and quantitative PCR demonstrate that these micro RNAs are overexpressed in the p27-null lymphomas harboring an activating provirus in Xq26. Given these findings, the Xq26 micro RNA cluster is a putative oncogene that may collaborate with p27-loss in tumorigenesis.

Design: To further examine the function of the Xq26 micro RNA locus and identify possible gene targets for the micro RNAs within this cluster, we have performed cDNA microarray analyses on our set of mouse lymphomas. RNA from 3 Xq26 positive lymphomas and 4 Xq26 negative lymphomas was isolated. After labeling, samples were hybridized to mouse expression arrays, scanned, and expression profiles generated.

Results: Expression data was normalized and the expression profile of Xq26 positive tumors was compared to Xq26 negative tumors. Global gene expression analyses revealed that the greatest fold differences (>2) occurred in four functional classes of genes involved in protein biosynthesis, rRNA processing, protein kinase regulation, and tRNA metabolism. Hierarchical clustering analysis revealed an expression signature for the Xq26 positive tumors that was distinct compared to the non Xq26 tumors. Examples of genes specifically found to be downregulated and therefore candidates for Xq26 micro RNA regulation included kinases (Kist, log ratio-1.5), phosphatases (Dusp4, log ratio-1.3), and cell cycle regulatory genes (Ccnd1, log ratio -.737).

Conclusions: The Xq26 micro RNA cluster is overexpressed in experimentally derived p27-null lymphomas. Gene expression analyses of Xq26 positive lymphomas demonstrate alterations in pathways regulating protein synthesis, rRNAs, tRNAs, and protein kinases. Regulatory components and genes in these pathways may be Xq26 micro RNA targets and the alteration of these pathways may collaborate with p27kip1-loss in human tumorigenesis.

1417 Evaluation of the Tumor Microenvironment for Metastasis (TMEM) and Its Associated Invasion Signature in Human Breast Carcinoma

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Background: Multiphoton-based intravital imaging (IVI) has demonstrated that carcinoma cells of living mouse metastatic tumors exhibit chemotaxis toward blood vessels. A paracrine loop involving tumor cells secreting CSF-1 and macrophages secreting EGF contributes to this directed movement. Ultimately, macrophages and tumor cells converge around blood vessels. The paracrine loop creates a microenvironment that leads to the expression of genes that enhance EGF-dependent chemotaxis and metastasis. This tumor microenvironment for metastasis (TMEM) and its associated expression profile, the invasion signature, has been defined for mouse PyMT tumors of the mammary gland. Mena is a key master gene in the invasion signature. The purpose of this study was to look for correlates of IVI observations in human breast cancer samples.

Design: Using double stain immunohistochemistry, we assessed the number and distribution of macrophages (CD68) and blood vessels (CD31) in sections from human breast tumors. Four compartments were evaluated: fat, non-neoplastic breast, DCIS, and invasive carcinoma. For each compartment, 70, 100um x 100um fields were evaluated. We also evaluated by immunohistochemistry the expression and localization of Mena.

Results: The numbers of blood vessels and macrophages are increased in breast tissue containing carcinoma. The highest density of macrophages in DCIS is at the epithelial-stromal interface. The highest macrophage and blood vessel densities are in invasive carcinoma ($P<0.01$). Mena expression is seen only in DCIS and invasive carcinoma and not in non-neoplastic breast tissue. The distribution of Mena is variably peripheral or localized to cell protrusions. Double staining with Mena and CD68 permits identification of TMEM.

Conclusions: The numbers of macrophages and blood vessels increase in invasive carcinoma and are seen in areas of Mena expression. TMEM is definable as close proximity of a tumor cell and macrophage to a blood vessel. Antibodies against Mena and macrophage markers are predicted to be useful as prognostic markers for metastatic disease. A retrospective analysis of cases with known outcome is underway.

1418 The Stem Cell Marker Nestin Identifies Aggressive Human Carcinomas and Promotes Motility and Metastasis

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Background: The intermediate filament protein Nestin identifies stem cells in the central nervous system and in CNS cancers, yet the biologic role of nestin is unknown. We previously identified nestin expression in carcinomas. Here we elucidate the significance of nestin expression in these common lethal cancers.

Design: Nestin expression was measured in cell lines and in surgical and autopsy cases. Short hairpin interfering RNA was used to knock down Nestin expression in cancer cells, which were assayed for growth, motility and invasion *in vitro* and local and metastatic growth *in vivo.*

Results: We detected nestin mRNA in human prostate, breast, and small cell lung cancer cells. In clinical samples, we found a striking association between nestin expression and clinically aggressive phenotypes. In breast cancer, nestin expression was found in high grade tumors, including those with anaplastic differentiation. In prostate cancer, we detected nestin protein in nealry 80% of lethal cases (n=32), but not in localized tumors (n=26), nor in pelvic lymph node metastases (n=32). Matched primary and distant metastases (n=9) showed concordant nestin expression in more than half of cases. We characterized growth, migration, and invasion in the AT6.3 rodent model of prostate cancer metastasis. Levels of nestin transcripts were 100-fold higher in highly metastatic AT6.3 cells than in benign NRP152 prostate epithelial cells, and were associated with markers of epithelial-to-mesenchymal transition (EMT). Nestin knockdown by siRNA showed no effect on growth, but inhibited cell migration and invasion. Compared to controls, tumors stably expressing nestin siRNA grew unabated at the site of inoculation, but were 5-fold diminished in the ability to form metastases.

Conclusions: Cell motility is a known but infrequently studied characteristic of stem cells. These results specify a function for nestin in cell motility and link this role to aggressive tumors. Gain of motility and loss of epithelial restraints are hallmarks EMT, considered a crucial step for metastasis. We propose that nestin contributes to EMT. EMT may be selected for at metastatic sites, or, as supported by our data, may originate at the site of origin, potentially identifying lethal cancers before they have metastasized. Thus, further characterization of nestin function may lead to strategies to neutralize metastatic spread, while measuring nestin expression may prove useful in predicting clinical course and tailoring therapy accordingly.

1419 Over-Expression of Transcription Factor SP1 in Pancreatic Adenocarcinoma

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Background: SP1, as a common transcriptional factor, is present at the promoter region of vascular endothelial growth factor (VEGF) that has been implicated as a key factor in tumoral angiogenesis in pancreatic adenocarcinoma. It is not known about SP1 expression and regulation of VEGF in the pancreatic cancer. In this study, we investigate expression of SP1 and co-expression of VEGF and SP1 in pancreatic andenocarcinomas.

Design: Eighteen cases of pancreatic ductal adenocarcinoma are collected from the archives of our institution. Immunohistochemical stains for both SP1 and VEGF in adjacent sections are performed using established protocol at our institution. The overexpresion is measured by clear stronger staining on tumor cells than that of benign ductal cells.

Results: The table below summarized the results. Briefly, tumors in nine of the eighteen cases overexpressed the VEGF; tumors in ten of the eighteen over-expressed the SP1. Among them, 8 out of 18 tumors overexpressed both VEGF and SP1 concurrently.

Conclusions: Overexpression of SP1 was found in pancreatic adenocarcinoma. Majority of SP1 positive tumors overexpressed concurrently with VEGF. Since function of SP1 naturally will increase the gene transcription of VEGF, our results suggest that the overexpression of SP1 in pancreatic cancer may play an important role in the up-regulation of VEGF expression.

Overexpression of VEGF and SP1 in pancreatic ductal adenocarcinoma

	Number of Cases (%)		
overexpression	VEGF	SP1	VEGF+SP1
	9/18 (50%)	10/18 (56%)	8/18 (44%)

1420 Mutational Profile of Sentinel Lymph Node Metastasis in Breast Cancer: Pathobiologic Implications

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Background: Breast cancer (BC) stage is the strongest predictor of disease aggressiveness and survival. Sentinel lymph node (SLN) has assumed a critical role in determining the need for axillary resection and additional therapy. The underlying mechanism for early tumor spread is largely unknown. We studied SLN spread using a combined histopathologic/molecular approach to gain insights into the pathobiology implications.

Design: Fourteen patients with positive SLN and BC were retrieved. Multiple microscopic tissue targets were microdissected from both primary and SLN for comparative mutational analysis. Aliquots of DNA were analyzed for allelic imbalance (LOH) using PCR/electrophoresis and a panel of 17 polymorphic microsatellite markers targeting 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, and 22q. Temporal sequence of mutation acquisition was based on clonal expansion model correlated with topographic distribution and extent of imbalance. Fractional mutation rate (FMR), a measure of cumulative acquired mutations, was defined as the total # mutations/total # informative markers.

Results: BC ranged from 0.5 to 8.0 cm in size. FMR ranged from 0.10 to 0.67 without consistent correlation with tumor size supporting that some BC can arise intrinsically as an aggressive malignancy with metastatic disease. The number of SLN removed ranged from 3-13 with 1-5 positive lymph nodes. The patients with higher FMR had higher positive SLN rate, while those patients with lower FMR had lower SLN positive rate.

Conclusions: By defining and then comparing the mutational profile between primary BC and SNL metastasis, valuable pathobiologic information concerning the intrinsic aggressiveness of BC can be determined that can be integrated with the histopathologic features. Higher FMR of BC which correlated with a greater number of positive SLNs may represent an aggressive subset of BC's having more extensive spread of disease. In addition, progressive accumulation of mutations in BC cases also was associated with the extent of regional SLN metastasis.

1421 Scaffold Proteins or Multi-Adaptors Proteins in Human Tumors. RACK1 Is Overexpressed in Lymphomas and Colon, Prostate and Breast Carcinomas

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Background: Cellular transformation requires activation of many oncogenic signals with a complex and as yet not well defined cross-talk among the different biochemical pathways. The presence of scaffold proteins or adaptors can connect several signaling enzymes, cytoskeletal elements and drive their location to several organelles in the cell. RACK1 is associated with PKC, phospholipase Cg, ras-GAP, some phosphatases, s-src, integrins and others. We hypothesized that scaffold proteins could be up regulated in human tumors and, consequently, associated with tumor progression.

Design: We analyzed RACK1 expression in a series of tumors including 116 colorectal carcinomas (CC) – including metastatic nodes when present-, 55 prostatic carcinomas (PC), 60 breast carcinomas (BC) and 35 high grade lymphomas -30 DLBCL and 5 FL grade 3-. We have also evaluated RACK1 expression in normal tissue and preneoplastic lesions such a colonic adenomas (62) and HGPIN areas (from the 55 PC, from 16 additional bladder carcinomas with HGPIN and no PC and in 76 needle biopsies). RACK1 immunohistochemical staining were performed in TMAs using monoclonal antibody. Positivity was semiquantitatively scored, including intensity (from 0 to 3) and percentage of positive staining. We obtained the HScore (range from 0 to 300) for statistical analysis. Clinical follow-up was available for all cases.

Results: RACK1 expression was increased in CC versus adenomas ($p<0.001$). No differences were found between adenomas and normal mucosa ($p=0.055$). High over-expression of RACK1 was associated with high-grade CC ($p=0.045$) and the presence of lymph node metastases ($p=0.026$). PC and HGPIN associated-carcinomas had RACK1 over-expression ($p=0.001$). In the prostate needle biopsies, high level of RACK1 was a good predictive factor of progression to PC (PPV of 100%). Finally, RACK1 over expression was observed in 83% of lymphomas and in over 80% of high grade breast carcinomas.

Conclusions: Scaffold proteins or multi-adaptor proteins like RACK1 are over expressed in human tumors. RACK1 associates with pathological grade in breast and colon carcinomas. Importantly, in HGPIN, high levels of RACK1 associates with carcinomas and, in needle biopsies, is a good predictor of progression to carcinoma in subsequent needle biopsies. Scaffold proteins can orchestrate several cellular pathways and may reflect the real oncogenic tumor potential, being therapeutic target candidates.

1422 The *Ataxia-Telangiectasia* Gene Product Is Required for Cellular Resistance to Transition Metal Toxicity

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Background: Iron and copper are significant sources of cellular oxidative stress due to their abilities to convert relatively non-reactive oxygen species such at H_2O_2 into the highly toxic hydroxyl radical. The *ataxia-telangiectasia mutated* gene product (ATM) is known to play a significant role in cellular resistance to oxidants. ATM is mutated in the rare progeric syndrome *ataxia-telangiectasia* (A-T), a disease characterized by cerebellar ataxia and elevated cancer incidence. Based on this, we hypothesized that ATM expression would increase cellular resistance to labile iron and copper toxcity.

Design: Normal, A-T, and A-T cells expressing recombinant ATM (rATM) sensitivity to iron, copper and iron and copper clelators was measured via colony forming assay. ATM kinase activation was measured via western blot examining ATM ser-1981 phosphorylation (a measure of ATM kinase activity). Additionally, transferrin receptor levels were examined via western blot in A-T cells and the same cells expressing rATM. Last, the effect of intra-abdominal desferal injection was examined in wild-type and syngeneic Atm-deficient mice was examined.

Results: When normal, A-T, and A-T cells expressing rATM were exposed to iron, copper, and iron and copper chelators the A-T cells, but not normal cells, exhibited a preferential sensitivity to both metal toxicities. rATM expression in A-T cells also increased A-T cell metal resistance. Chelators of both metals also increased A-T cell, but not normal cell growth, +/- exogenous peroxide exposure. Both metals and the iron chelator desferal also activated ATM kinase activity. Additionally, transferrin receptor expression was increased with in A-T cells compare to the same cells expressing rATM. Last, desferal treatment increased the weight of Atm-deficient mice, while lowering the weight of syngeneic wild-type mice.

Conclusions: ATM expression plays an important role in cellular resistance to the toxic effect of the transition metals iron and copper. Additionally, our data demonstrating the unusual responses of A-T cells to both iron and copper chelators, the dysregulated transferrin receptor expression in A-T cells, and the differential effect of desferal upon the weights of Atm-deficient vs. syngeneic wild-type mice indicates that ATM plays a role in both transition metal metabolism and resistance to the pro-oxidant effects of transition metals.

1423 Immature Tumor Infiltrating Dendritic Cells Are Increased in Lymph Node-Positive Colorectal Carcinomas Compared to Node-Negative Tumors

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Background: Immature tumor infiltrating dendritic cells (TIDC) engulf dying tumor cells and apoptotic tumor cell debris, process antigens, and migrate to regional lymph nodes, where they mature and engage an emergent clone of naive T cells bearing the correct complementary specificity. Although one may consider the presence of numerous TIDC to reflect a robust host immune response and, thus, represent a favorable prognostic factor, recent data suggest that higher numbers of TIDC are associated with poor clinical outcome among patients with colorectal carcinoma (CRC). The mechanisms underlying this seemingly counterintuitive observation are unknown. We hypothesized that increased immature TIDC in CRC may be a surrogate marker of regional lymph node metastases. Therefore, the aim of this study was to determine whether the presence of TIDC in CRC was associated with pathologic tumor stage or any other clinical or pathologic feature.

Design: The study group consisted of 25 surgically resected CRC (10 stage II, 10 stage III, 5 stage IV) that were pathologically staged in accordance with the AJCC Cancer Staging Manual, 6th edition. All 15 stage III or IV tumors had N1 (7) or N2 (8) disease. The tumors were evaluated for grade, tumor infiltrating lymphocytes (TIL), and a Crohn's-like response. Tissue sections of the tumor were immunostained with antibodies to CD1a to identify immature TIDC, as well as CD83, a marker of mature TIDC. The TIDC density was calculated as the mean of 35 high power fields.

Results: The mean age of the patients was 70 years [male/female: 3/2]. There were no differences in pathologic grade or the presence of a Crohn"s-like response among the groups, but all 4 cases with TIL were stage III (3), or IV (1, $p>0.05$ for all comparisons). The density of CD1a+ TIDC was significantly lower in stage II tumors (mean: 0.28), compared to stage III (mean: 2.4, $p=0.001$) and IV (mean: 2.7, $p=0.003$) tumors. Similar numbers of mature CD83+ TIDC were seen in stage II, III, and IV tumors (mean: 0.41, 0.20, 0.27, respectively, $p>0.05$ for all comparisons). No association between TIDC density and any other clinical or pathologic feature was noted.

Conclusions: The number of immature CD1a+ TIDC present in CRC increases with progressive stage. Numerous TIDC are significantly associated with the presence of lymph node metastases. Further studies investigating the role of immature TIDC in colorectal carcinoma nodal metastasis are warranted.

1424 Elevated Expression of Tumor Aggressiveness and Invasiveness Related Proteins in Cells Overlying Focally Disrupted Myoepithelial Cell Layers

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Background: Our previous studies in ductal carcinoma *in situ* (DCIS) revealed that a subset of these tumors contained focal disruptions in surrounding myoepithelial (ME) cell layers (the absence of ME cells resulting in a gap greater than the combined size of at least 3 ME cells). Cells overlying these focal disruptions had significantly higher rate of genetic instabilities and expression of tumor invasion-related genes than adjacent cells within the same duct (Man et al. Breast Cancer Res 5:231-241, 2004; Man et al. Breast Cancer Res Treat, 89:199-208, 2005). This study attempted to assess the expression status of tumor aggressiveness- and invasiveness- related proteins in these cells.

Design: Consecutive sections were made from 50 breast tumors with co-existing normal, hyperplastic, *in situ*, and invasive components. Sections were immunostained for smooth muscle actin to elucidate ME cell layers, and for c-erbB2 and CAPC, which are associated with tumor aggressiveness and invasiveness (Egland et al. Proc Acad Natl Sci USA 103: 5929-5934, 2006; Man et al. Nova Science Publishers, Inc, in press). The expression levels and frequencies of these molecules in cells overlying focal ME cell layer disruptions, adjacent cells within the same tumor, and invasive cancer cells were statistically compared. In 5 selected cases, these cells were microdissected and the mRNA levels of c-erbB2 and CAPC were quantitatively compared.

Results: A subset of DCIS and normal and hyperplastic ducts contained focally disrupted ME cell layers. Compared to their adjacent counterparts within the same duct, cells overlying these focal disruptions consistently showed several unique features: [1] substantial alterations in cell size, shape, density, and polarity; [2] consistent co-expression of high levels of c-erbB2 and CAPC proteins; [3] markedly increased mRNA levels of c-erbB2 and CAPC. These changes were similar to those seen in adjacent invasive cancer cells, except that c-erbB2 expression seen in cells overlying focal ME cell layer disruptions was consistently present in the cytoplasm.

Conclusions: The substantial differences to adjacent cells within the same tumor and similarities to invasive cancer cells suggest that cells overlying focal ME cell layer disruptions may represent the direct precursor of invasive lesions (Supported by grants DAMD17-01-1-0129, DAMD17-01-1-0130, and PC051308 from Congressionally Directed Medical Research Programs to Yan-gao Man, MD., PhD).

1425 Inconsistent Expression Pattern of BRM Chromatin Remodeling Complex across Human Cancers and Its Clinical Significance

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Background: Chromatin remodeling complexes have been the subject of strong interest in human cancers. Previously we reported BRM is a prognostic factor in lung cancer and may not compose uniform complex with other known subunits of BRM (hSwi/Snf) complex. Known BRM complex includes BRM, RbAP48, BAF155, and BAF170. However, little is known about consistency or variation of their expression across various human cancers.

Design: We immunohistochemically investigated the expression of 4 components of BRM complex using tissue microarray with 1150 cases from 14 major cancers; containing 200 lung cancer cases, 100 cases of breast, kidney, bile tract, thyroid, liver, colon, and stomach cancer, and 50 cases of prostate, pancreas, bladder, ovary, and uterine body cancer. Nuclear signal of these proteins were correlated each other and with patients' clinical data.

Results: The positive rate of BRM, RbAP48, BAF155, and BAF170 were as indicated in table1. In total cohort, expressions of all four molecules correlate each other. ($p<0.01$) BRM expression negatively correlate with pathological stage ($p=0.033$). BRM also correlate with gender (F>M, $p=0.025$). In lung adenocarcinoma, BAF155 negatively correlate with lymph node metastasis ($p<0.01$). BAF170 express consistently high in most of cancer types. However, expression of the other proteins differs. BRM express

significantly lower in lung adenocarcinoma and liver cancer and higher in breast cancer, BAF155 express significantly lower in liver and kidney cancer and higher in colon, stomach, and uterine body cancer, and RBAP48 express significantly lower in liver and prostate cancer. ($p<0.01$)

Conclusions: Expression of BRM complex subunits showed negative correlation with factors indicating cancer progression in various conditions, which is consistent with reported data. The expression rate of each subunit varies by cancer types. Although BAF170 express consistently high in most of cancer types, BRM, BAF155, and RBAP48 express significantly different compare to the average.

Table 1.

	BAF170	BRM	BAF155	RBAP48
ALL	97%	27%	72%	89%
LUNG SCC	94%	31%	85%	96%
LUNG AD	99%	**5%**	59%	91%
BREAST	97%	**60%**	86%	98%
KIDNEY	98%	**13%**	**35%**	92%
BILE TRACT	96%	**10%**	62%	92%
THYROID	97%	26%	68%	82%
LIVER	92%	**4%**	**33%**	**71%**
COLON	100%	**52%**	**98%**	99%
STOMACH	100%	29%	**90%**	94%
PROSTATE	96%	43%	83%	**71%**
PANCREAS	92%	**11%**	77%	100%
BLADDER	98%	**47%**	88%	90%
OVARY	93%	23%	81%	90%
UTERINE BODY	100%	24%	**95%**	98%

1426 Loss of Histone Acetyltransferase MORF Is a Less Common, but Specific Molecular Event in Cellular Leiomyomas

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Background: Cellular uterine leiomyomas (C-ULM) are less common (5% of leiomyomas) and are characterized by their cellular nature. A recent study identified a new reciprocal translocation of t(10;17) from several C-ULM. The translocation disrupts the histone acetyltransferase MORF. To evaluate if loss of MORF is a common molecular event, we examined expression of MORF mRNA in large numbers of usual and cellular type ULM.

Design: Ninety cases of uterine leiomyomata were studied, of which 60 cases were of the usual type and 30 cases of the cellular type. Triplet tissue cores from usual type and cellular leiomyomata along with 2 normal matched myometrial cores were collected and arrayed in tissue microarray. The probe NYMS4 (histone acetyltransferase MORF cDNA) was prepared and cloned into TOPO vector with SP6 and T7 promoters. Digoxin-labeled probes (sense and antisense) were synthesized using SP6 and T7 DNA polymerase. In-situ hybridization was performed according to a standard protocol.

Results: MORF cRNA probe was prepared from 5' coding region. Expression of MORF mRNA was detectable with moderate intensity by in situ hybridization in both ULM and matched myometrium. In MORF mRNA positive cases, intensity of MORF mRNA was higher in ULM than that in the matched myometrium. No difference of MORF mRNA was found between usual type and cellular type. Among 60 usual type ULM, no loss of MORF mRNA was identified. However, in 3 cases out of the 30 cellular leiomyoma (10%), complete loss of MORF mRNA was present in all 3 tumor cores, but not in matched myometrium.

Conclusions: Expression of MORF mRNA is detectable in both myometrium and usual type of leiomyoma. The level of MORF mRNA expression tends to be higher in ULM. Loss of MORF is observed in 10% of C-ULM but 0% in the usual type ones. Loss of MORF is less common but specific to C-ULM, suggestive of a unique tumorigenic role of MORF in C-ULM.

1427 Expression Profiling of Colonic Epithelium by Laser Capture Microdissection Proposes a Role for Rantes (CCL-5) in the Pathophysiology of Lymphocytic Colitis

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Background: Lymphocytic colitis (LC) is a disorder characterized by increased numbers of T-lymphocytes infiltrating colonic epithelium. Although the histopathological features of LC are well characterized the pathophysiology of this disorder remains uncertain. Our aim was to determine whether expression profiling can identify inflammatory mediators contributing to the pathophysiology of LC.

Design: Paraffin blocks from cases of LC, collagenous colitis(CC) and aged matched controls with a history of diarrhea but histologically normal (NC) were retrieved from the pathology archive of the Rhode Island Hospital. For IHC, intraepithelial T-lymphocytes infiltrating the surface epithelium were stained with anti CD3 antibody. T-cells were enumerated microscopically and expressed as CD3 labeled T-cells per 100 epithelial cells. For laser capture microdissection (LCM) surface epithelium which included the infiltrating lymphocytes was separated from the surrounding stroma by LCM. RNA was extracted and reverse transcribed using Paradise FFPE reagents from Molecular Devices, Sunnyvale CA. The resulting cDNA was used to probe a Th1,Th2,Th3 RT^2 Profiler PCR Array (SuperArray Bioscience Corp, Frederick, MD) for the genetic expression profile of key genes involved in the inflammatory immune cascade.

Results: Six Profiler PCR arrays were run for each subgroup (6LC, 6CC, 6NC). Ten genes were upregulated 2 fold or more in the epithelium from laser-captured lymphocytic colitis as compared with normal colonic epithelium (CCL5, CCR4, GFI1, IGSF6, IRF4, JAK2, LAT, PCGF2, STAT1, TNFRSF9) whereas seven were up regulated in collagenous colitis (CCL11, CCL5, GATA3, IL5, LAT, SOCS2, TNFRSF9).

Upregulated Genes in Lymphocytic Colitis

Gene	RANTES	CCR4	GFI1	IRF4	PCGF2	STAT1	TNFRSF9
Fold Increase	12.48	4.66	2.35	2.31	2.55	2.42	2.82

Upregulated Genes in Collagenous Colitis

Gene	RANTES	CCL11	GATA3	IL5	LAT	SOCS2	TNFRSF9
Fold Increase	2.76	2.40	2.23	2.55	2.08	2.13	2.38

Conclusions: Expression profiling by LCM of archival pathology specimens is a novel technique by which to define new inflammatory mediators involved in the pathophysiology of microscopic colitis.

1428 Differential Expression of Myocyte Enhancer Factor (MEF2) Isoforms in Rabdomyosarcoma

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Background: Recent studies showed that MEF-2 isoforms (MEF2A, B, C and D) played different roles in muscle differentiation and neuronal maturation. MEF-2A and 2D have been shown to specifically promote differentiation and survival of cerebellar granular cells. MEF-2C-null mice are embryo lethal, while MEF-2A-null mice develop cardiac myopathy. MEF2 protein has also been detected in cardiac myxoma and rhabdomyosarcoma by using non-isoform specific MEF2 antibody. However, the expression of MEF-2 isoforms in myogenic or neurogenic tumors is unclear. This study evaluates the histologic expression pattern of MEF2 isoforms in rhabdomyosarcoma and myxoma by using immunohistochemistry with isoform-specific MEF2 antibodies.

Design: Rhabdomyosarcoma, cardiac myxoma, intramuscular myxoma, neuroblastoma and Ewing's sarcoma/PNET (primitive neuroectodermal tumor) cases were reviewed. Immunostaining with MEF2A-, MEF2C-, MEF2D-specific antibody and isoform non-specific antibody were performed with proper positive and negative controls. The stain results (intensity) were assessed by two pathologists and graded as positive (++), weak positive (+) and negative (-). The stain distribution was also evaluated as extensive (>50% of the cells) and patchy (<50% of the cells). The human left atrium is served as positive control.

Results: All the MEF2 isoform-specific and non-specific antibodies positively stained the nuclei in the control and positive cases. There is no cytoplasmic expression identified. The detailed results are listed in the table.

Conclusions: MEF2 proteins are differentially expressed in embryonal and alveolar types of rhabdomyosarcoma and cardiac myxoma but not PNET and neuroblastoma. However, only MEF2A and 2D isoforms are expressed in rhabdomyosarcoma cells while only MEF2C was expressed in myxoma cells. Our data suggests that MEF2 isoforms are differentially involved in the myogenic differentiation of rhabdomyosarcoma and cardiac myxoma, and the MEF2 antibodies may provide a new marker option in the differential diagnosis of rhabdomyosarcoma against Ewing's sarcoma and neuroblastoma.

Table. Results of Immunostains

Case	Musc. spe actin	Desmin	MEF2A	MEF2C	MEF2D	MEF2 non-spec
Rhabdo. Embryon	+	+	P++	-	P+	++
Rhabdo. Alveolar	+	+	P+	-	P+	++
Rhabdo. Botryoid	+	+	-	-	-	-
Cardiac myxoma	n	n	+	+	+	+
Intra-musc. myxoma	n	n	+	+	+	+
Ewing's/PNET	n	n	-	-	-	-
Neuroblastoma	n	n	-	-	-	-

P: patchy; N: not determined; ++:positive; +:weak positive

Pediatrics

1429 Clinicopathologic Study of the Liver of Children with Sickle Cell Disease

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Background: Children with sickle cell (SC) anemia undergo chronic blood transfusion (CBT) therapy that may lead to liver injury. The aims of this study were to correlate histopathologic changes in liver biopsies from SC children with clinical data, laboratory values and number (no.) of transfusions.

Design: The aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), serum ferritin (SF) and the no. of transfusions received up to the time of biopsy were recorded. Liver injury was assessed histologically by architectural disruption; inflammatory activity grading and fibrosis (modified Scheuer system). Pathologic grading of liver siderosis was assessed by intensity of Prussian blue staining (1-4+; 1+ is minimal focal staining, mostly Kupffer cell; 4+ is strong diffuse, definitive hepatocyte staining). TB and SF levels were correlated with no. of transfusions and severity of liver siderosis and SF were correlated using Pearson and one-tailed t-test.

Results: 18 liver biopsies from 11 patients (6 female, 5 male; 6-22 years old) were analyzed. All patients were free of viral hepatitis. The mean no. of transfusions was 42 units. AST ranged from 19-66 IU/L; ALT from 12-37 IU/L; TB from 1.1-7.9 mg/dl and SF from 717-7959 ng/ml. There was no significant correlation between TB and SF levels with increasing no. of transfusion ($R = 0.073$, p value = 0.385; $R = -0.018$, p value = 0.253 respectively). All the liver biopsies showed preserved lobular and bile duct architecture. Inflammation was present in 8 cases, 2 with grade II inflammation, 6 with grade I inflammation. 12 cases showed stage 1 fibrosis. Iron scoring was1+ (n=1), 2+ (n=7), and 3+ (n=6). There was a significant correlation between the SF level and the histopathologic grade of liver siderosis ($R = 0.598$, p-value = 0.004). Kupffer cell hyperplasia was noted in 6 (33%) cases; glycogenation of the nuclei in 7 (38.9%); extramedullary erythropoeisis in 2 (11%). Serial biopsies were available from 6 patients and showed no progression of fibrosis after a range of 11 – 71 units of transfusion.