### 1367 Colorectal Intramuscal Perikarya of Ganglion Cells

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**Background:** It is generally believed that perikarya of ganglion cells in the normal human colonic mucosa are confined to the muscularis propria. Intramucosal perikarya of ganglion cells have been noted only in ganglioneuromas, neuronal intestinal dysplasia, and the appendix.

**Design:** Retrospectively reviewed 58 specimens from colorectal biopsies. For each specimen, the presence of intramusical perikarya of ganglion cells, their number, location and grouping were recorded, as well as the diagnoses. Immunostains for neuron-specific enolase and cytomegalovirus were performed to attempt to confirm the presence of intramusical perikarya.

**Results:** Eleven specimens (19%) contained intramusical perikarya. Intramusical perikarya were located throughout the large intestine and occupied the muscularis propria. Intramusical perikarya were seen in normal mucosa, hyperplastic polyps, adenomas, carcinoma, inflammatory bowel disease, and cytomegalovirus-associated colitis.

**Conclusions:** We present the first study of colorectal intramusreal perikarya of ganglion cells. Our findings demonstrate that intramuscal perikarya of ganglion cells are present in normal and abnormal mucosa. Awareness of intramuscal perikarya is necessary to avoid confusion with microgranulomas or cytomegalovirus.

### 1368 Uveal Melanoma and Monosomy of Chromosome 3

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**Background:** Risk assessment for uveal melanoma currently uses attributes such as tumor size and histologic cell type. In the future, morphologic molecular pathology may complement or replace those attributes. Monosomy of chromosome 3 has been associated with an adverse outcome. Fluorescence in situ hybridization (FISH) was used to evaluate uveal melanomas for monosomy of chromosome 3.

**Design:** A tissue microarray was constructed using two or more cores of uveal melanomas from 15 patients. FISH using a directly labeled probe specific for the pericentrimeric region of chromosome 3 was performed. Tumor cell nucleli were evaluated under a high magnification. FISH was successful in 13 tumors. 8 tumors revealed monosomy 3. 5 had normal 3. 2 tumors were diploid. 1 showed supernumary copies. 1 showed trisomy 3. 4 tumors had a normal chromosome 3.

**Results:** FISH was successful in 13 tumors. 8 tumors revealed monosomy 3. 5 had normal 3. 2 tumors were diploid. 1 showed supernumary copies. 1 showed trisomy 3. 4 tumors had a normal chromosome 3. Of the 8 tumors with monosomy 3, 7 became metastatic at one, three and six years. 1 is dead of disease. All of the patients developing metastatic disease had chromosome 3 monosomy. All 5 patients with tumors with 3 monosomy had no evidence of disease at last follow up that varied from one year to five years. 5 patients with monosomy 3 were alive without evidence of disease at zero to eight years follow up.

**Conclusions:** FISH for chromosome 3 monosomy can complement other attributes used in risk assessment of uveal melanoma.

### 1369 Differential Amplification and Expression of CDK6 between Low-Grade Astrocytoma and Glioblastoma Multiforme

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**Background:** Astrocytomas are the most common primary brain tumors occurring in the adult central nervous system. Emerging evidence suggests that the development of glioblastoma multiforme (GBM) is a multi-step process and is result of a series of genetic alterations occurring over time. Our previous study using array-based comparative genomic hybridization (A-CGH) demonstrated marked difference in the extent of genomic gains and losses between GBM and low-grade astrocytoma (LGA). In this study, we validated the A-CGH results for cyclin-dependent kinase 6 (CDK6) copy number changes by fluorescence in situ hybridization (FISH) and examined the expression level of CDK6 protein between GBM and LGA by immunohistochemistry (IHC).

**Results:** A tissue micro-array (TMA) containing 31 GBM and 5 LGA was constructed and used for FISH and IHC. The CDK6 probe derived from a bacterial artificial clone (BAC) was labeled with SpectrumGreen dUTP. The CDK6 and CEFP probes were hybridized with the TMA slides. Immunostaining on TMA was performed using monoclonal antibody specific for CDK6.

**Results:** Among the 15 cases of GBM, 10 cases were positive for CDK6. 5 had increased copy number of CDK6, whereas all five cases of LGA displayed no changes. 5 cases of GBM exhibiting high-level amplification also showed strong immunostaining for CDK6 and the other 9 cases were moderately positive. Among the cases with no copy number changes for CDK6, 5 cases of GBM stained weakly to moderate. Immunostaining on TMA was performed using monoclonal antibody specific for CDK6.

**Conclusion:** The differential changes in CDK6 copy number between GBM and LGA. Overexpression of CDK6 protein resulting from either increased the copy number or enhanced transcription/translation may play a role in the development of GBM and the possible progression of low-grade astrocytomas to GBM. In addition, our study demonstrates that array-based genomic profiling together with FISH validation and IHC can be effective tools for the identification of new molecular markers having potential clinical utility.

### 1370 Diagnostic Utility of Epithelial Markers in Separating Choroid Plexus Papillary Neoplasms from Metastatic Carcinomas with Papillary Architecture

**VX Sun, YJ Liu, M Tang, HP Silverman. Allegheny General Hospital, Pittsburgh, PA.**

**Background:** Choroid plexus papillary neoplasms (CPPs) are epithelial tumors derived from the choroid plexus (CP). The diagnosis of choroid plexus papillary neoplasms is usually not difficult based on the histologic and clinical features. However, in patients with a history of a papillary carcinoma, separation of choroid plexus papillary neoplasm from metastatic carcinoma with papillary features is very important and can be occasionally challenging. The utility of immunohistochemical markers to separate choroid plexus papillary neoplasm from metastatic carcinoma with papillary features has not been investigated.

**Design:** A total of 38 cases including 11 normal choroid plexus, 8 choroid plexus papillary neoplasms and 20 metastatic carcinomas with papillary features metastatic to the central nervous system were reviewed. Epithelial markers of CAM5.2, CK7, CK20, CK5/6, Ber-EP4, EMA, and B72.3 were evaluated. Immunostains were performed on a automated immunostainer with appropriate positive and negative controls. Statistical analysis was calculated with Chi-square method.

**Results:** Expression of epithelial markers in choroid plexus papillary neoplasms (CPPs), normal choroid plexus (CP)and metastatic carcinomas with papillary architecture

**Markers:**

- CAM5.2
- CK7
- CK20
- CK5/6
- Ber-EP4
- EMA
- B72.3

**PPCs:**

| Marker | Expression |
|--------|------------|
| CAM5.2 | 100% (6/6) |
| CK7    | 100% (6/6) |
| CK20   | 100% (6/6) |
| CK5/6  | 100% (6/6) |
| Ber-EP4| 100% (6/6) |
| EMA    | 100% (6/6) |
| B72.3  | 100% (6/6) |

**Conclusions:**

1) Ber-EP4 and B72.3 are expressed in 100% and 70% of metastatic carcinoma with papillary features but none of the choroid plexus papillary neoplasms. Therefore, Ber-EP4 and B72.3 are useful immunohistochemical markers in separating metastatic carcinoma from CPPs.

### 1371 Persistent Increase of Immature Reticulocyte Fraction in Sickle Cell Anemia Treated with Hydroxyurea

**R Bagdasaryan, F Chaves, K Quillen, M Gallinaro, D Xu. Boston University School of Medicine, Boston, MA.**

**Background:** Sickle cell anemia is an inherited disease. The deformed red blood cells obstruct the circulation causing severe pain, hemolytic anemia, and other complications. Recent studies have shown that hydroxyurea (HU) increases the concentration of HbF that prevents the polymerization of HbS and significantly improves the microcirculation, leading to decrease in the number of vaso-occlusive episodes in patients with sickle cell anemia (Steinberg et al., 2003). Since it has been noted that the Immature Reticulocyte Fraction (IRF), a ratio of immature reticulocytes to the total number of reticulocytes, is markedly increased in sickle cell anemia, we investigated the effect of HU on IRF, which serves as an important physiologic indicator of the microcirculation.

**Design:** Thirty-one patients with sickle cell anemia treated at Boston Medical Center, Boston, MA were studied, including 16 treated with HU (mean age = 24 years, M = 12.8) and 16 without HU (mean age = 13 years, M = 10.0). Laboratory observations include IRF as well as Hb, Hct, MCV, reticulocyte %, absolute reticulocyte count (ARC) by automated hematology analyzer (Beckman Coulter), and HbF by HPLC.

**Conclusions:** In all cases, peripheral blood smears were also reviewed. Statistical analysis was performed using student t test.

**Results:** The significant differences between HU-treated vs non-treated groups were observed in Hb (12.4% vs 8.0%; p<0.05), MCV (102.7 fL vs 92.2 fL; p<0.01). Even more dramatic differences in HbF (18.8%), reticulocyte % (5.8%) and ARC (1600) were noted in the treated group with MCV greater than 100 fL in 14 of 16 (87.5%) patients. The decrease in IRF was not statistically significant in the number of sickle cells per HPF in the
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peripheral blood smear in the higher MCV subgroup. No differences in Hb (9.4 ± g/dL vs 8.6 ± g/dL) and RBC (2.7 x 10^6 vs 2.7 x 10^6; p=0.37) were observed in treated group compared to non-treated group. The IRF was also similarly elevated in these two groups (0.60 vs 0.61; p=0.4) (normal: 0.16 – 0.36).

Conclusions: Although hydroxyurea has significant effects on the RBC in sickle cell anemia patients in INH MCV, and decreases in numbers of sickle cells, reticulocyte % and ARC, the IRF remains consistently elevated in treated patients, which may imply continuous bone marrow stimulation secondary to chronic tissue hypoxia. The pathophysiological mechanism needs to be further elucidated.

1372 Clinical Proteomics: In Vivo Molecular Signaling Profiles of Human Tumors, Pre and Post Tumor Perfusion with Experimental Chemotherapy KR Calvo, V Expnla, A Rodrigue, S Hoytson, EP Petricion, RH Alexander, LA Liotta. Dana-Farber Cancer Institute, La Jolla, CA.

Background: The future of cancer treatment lies in the development of individualized “designer” therapies tailored to the specific deranged molecular circuitry of an individual patient’s tumor within the tissue microenvironment. We have generated proteomic profiles of human tumors in patients, measuring tumor and host cell response to chemotherapy in vivo, in real time along multiple protein signaling networks regulating survival, proliferation, apoptosis and differentiation.

Design: Biopsies of tumor and adjacent tissue were taken during surgery pre and immediately post perfusion with high dose chemotherapy to capture in vivo proteomic signaling profiles. All patients were undergoing isolated hepatic perfusion (IHP) or isolated limb perfusion (ILP) for various metastatic cancer including melanoma, colorectal adenocarcinoma, and adenocarcinoma with unknown primaries of lymph nodes of permanent tumor and adjacent host cells were obtained via laser capture microdissection. Reverse phase protein microarrays were utilized to determine the expression of proteins within multiple signaling networks including: AKT, Caspase 3, Bax, Hifalpha, p38, cKit, EGF, Her2, ERBB2, Cox2, HSPT1, NFkappaB, FAK, PKC, eIF4E, STAT1, and STAT3.

Results: Proteomic profiles measuring the activity of over 18 proteins in signaling pathways including proteins known to be inactivated from small cell lung cancer, displayed uniquely different proteomic profiles and had measurably different molecular responses to perfused chemotherapy. In all cases evaluated to date, significant increases in phosphorylation events resulting from perfusion pathways were detected in response to therapy in tumor cells relative to host cell profiles.

Conclusions: For the first time, in vivo proteomic profiles of the molecular circuitry of human tumors and host tissue have been generated with measurement of the response to in vivo chemotherapy to perfused therapy. This provides a unique platform for the molecular analysis of human tumors in vivo with the identification of deranged protein signaling pathways suitable for targeting by combinatorial therapy with molecular inhibitors. Analysis of the molecular response of human tumors in vivo to therapy has broad applications for the strategic design of individualized molecularly targeted therapies.

1373 Expression of Hedgehog Signaling Pathway (SHH, PTCH, SMO) in Pancreatic Ductal Carcinoma and Pancreatic Intraductal Neoplasia (PanIN) Y Dancer, C Hoard, J Albores-Saavedra. Louisiana State University Health Science Center, Shreveport, LA.

Background: The Hedgehog (Hh) signaling pathway is crucial for normal development and patterning of numerous human organs including the pancreas. Sonic hedgehog (SHH) has recently been implicated as a crucial factor in pancreatic organogenesis and gland differentiation. Recently, dysregulation of these developmentally important genes has been implicated in cancer. In this study we analyze the expression of Hh signaling proteins in pancreatic ductal carcinoma.

Design: Normal pancreas, pancreatic ductal carcinoma and PanINs were examined by immunohistochemistry using antibodies against Hh signaling molecules and secreted protein Sonic hedgehog (SHH), in receptor Patched (PTCH), and the PTCH-associated transmembrane protein Smoothed (SMOH). Histologic sections from 28 pancreatic ductal carcinomas were stained with antibodies against SHH, PTCH and SMOH (Santa Cruz, CA). Ductal lesions associated with these tumor cases were classified as PanIN-1, 2, 3, 3, using newly defined criteria. Normal colonic tissue and pancreatic tissue from autopsy specimens were used for control. Interpretation of the results was done by three pathologists as follows: The expression of SHH, PTCH, SMOH was graded as positive when there was more than 5 percent of cytoplasmic staining.

Results: In our preliminary results, SHH was expressed in 17 of 28 invasive ductal carcinomas cases (61%). Progressively higher expression of SHH was observed in ductal epithelium of PanIN-1 to -3 and in the surrounding reactive mesenchymal cells. PTCH was expressed in the epithelium of the invasive ductal carcinoma, PanIN-1 to -3 and surrounding reactive mesenchymal cells in 20 of 28 cases (71%). However, the results of SMOH in some cases are still pending.

Conclusions: Hh signaling components: SHH and PTCH are strongly expressed in Pancreatic Ductal Carcinomas and PanINs. This study suggests that Hh signaling pathway may play a pivotal role in the initial development and progression of pancreatic ductal carcinoma.

1374 Distribution and Expression of Pancreatic Duodenal Transcription Factor 1 (PDX-1) in Bile Duct, Pancreas and Gastrointestinal Tract Mucosa C Deng, Y Lin, P Cho, M Tung, JF Silverman. Allegheny General Hospital, Pittsburgh, PA.

Background: PDX-1, a pancreatic duodenal transcription factor 1, plays essential roles in regulating the development and proliferation of pancreatic exocrine and endocrine cells. Impaired transcription of PDX-1 during early pancreatic development leads to agenesis of the pancreas. In adult, PDX-1 is restricted primarily to beta cells where it regulates the expression of a number of pancreatic genes, including insulin, somatostatin, islet amyloid polypeptide, the glucose transporter type 2 and glucokinase. The distribution and expression of PDX-1 in the normal bile duct, pancreas and gastrointestinal tract mucosa have not been investigated.

Design: Total 56 cases including 8 each from benign gastro-esophageal junction, gastro-esophageal junction mucosa with intestinal metaplasia, stomach, duodenum, bile duct, pancreas and colon were selected. Immunohistochemical study for PDX-1 was performed. Sections from pancreatic islet cells were used as positive control. Results: The immunoreactivity within the nuclei was counted as positive. Total of 28 cases were analyzed. Total 20 cases the PDX-1 stain was seen in the epithelial cells of gastro-esophageal junctional mucosa with or without intestinal metaplasia (16/16, 100%), epithelial cells of stomach (8/8, 100%), duodenum (8/8, 100%) and bile duct (8/8, 100%). In the pancreas, the stain was seen in the acinar cells and islet cells (8/8, 100%). No immunoreactivity was detected in any of the pancreatic ductal cells. Colonic mucosa showed only luminal cytoplasmic staining without nuclear reactivity.

Conclusions: PDX-1 expression was observed in the epithelial cells of gastro-esophageal mucosa with or without intestinal metaplasia, gastric and duodenal epithelium, benign biliary ducts and pancreatic acinar and islet cells. No PDX-1 immunoreactivity was detected in the benign pancreatic ductal cells. The pattern of distribution and expression of PDX-1 may be useful in elucidating the metastatic carcinoma of unknown origin.

1375 Pharmacodynamic Assessment of the Cyclin-Dependent Kinase Inhibitor Flavopiridol: Modulation of cdk Targets in Clinical Samples D Di Vizio, FP O’Connell, N Bhattacharya, GL Shapiro, M Ledda. Dana Farber Cancer Institute and Brigham and Women’s Hospital, Boston, MA.

Background: Cyclin-dependent kinase inhibitors (Cdki) have been postulated to have efficacy in a variety of cell types with overexpression of cyclin-regulated and/or cyclin-regulated protein kinases (Cdk). The most promising candidate is flavopiridol (FP). The pharmacodynamic assessment of FP from clinical samples is necessary. In this study, we analyzed the expression of cdk targets in clinical samples and their modulation by FP.

Design: Skin biopsies (n=12) or tumor sampling (n=2) were performed prior to and 2 hrs after the first infusion. Paraffin-embedded samples were analyzed by immunohistochemistry for effects of treatment on cdk targets including total and phosphorylated (cdk2 and cdk4), total and phosphorylated cdk7 (cdk2), and cdk5 and cyclin D1 (cdk9). Approximately 200 nuclei were routinely scored for 0, 1+ and 2+ staining; the sum of 1+ and 2+ staining was used to determine the % positive cells.

Results: Among 12 sets of paired skin biopsies, 6 demonstrated reduced staining of Rb [p54/249/252] post treatment (p=0.025) and 8 demonstrated decreased Rb staining at the [S807/811] phosphorylation site (p<0.002). Depletion of phospho-Rb occurs while staining for total Rb is preserved. In addition, increases in total p27 occur post-flavopiridol, consistent with decreased [pT187] phosphorylation. Consistent with inhibition of cdk9, the majority of paired skin biopsies demonstrated either stable or increased p53 levels post-treatment. Reduced staining of cyclin D1 was also demonstrated. In the two patients with accessible tumor tissue, staining demonstrated decreased phospho-cdk7, increased total p27 and decreased phospho-Rb [p5807/811] post treatment.

Conclusions: These data demonstrate that flavopiridol can induce biologic effects in tumor and surrogate proliferating tissue.

1376 Molecular and Morphological Correlates in Lung Cancer M Edgerton, LJ Frey, DH Fisher. Vanderbilt University Medical Center, Nashville, TN.

Background: Current therapeutic options for patients with non-small cell lung carcinoma are based upon histological subtype, grade, and stage of the tumor. Analysis of histopathological morphologies by pathologists is the gold standard for diagnosis, grade, and pathological staging of tumors. Gene expression profiles that predict morphology can have causal relevance for the patient’s outcome.

Design: Published cDNA gene expression array data for 34 non-small cell lung carcinomas was used for this data mining application. The cases were distributed as 15 squamous cell carcinomas (SCLC), 11 adenocarcinomas (AC), and 8 large cell undifferentiated (LG) carcinomas. The grade distribution was 12 moderately (6 AC and 6 SCLC), 14 (5 AC and 9 SCLC) poorly, and 8 undifferentiated. Error rates were estimated using leave one out cross validation. The algorithm C5.0 was used to generate decision trees to predict differentiation (grade) from expression data in combination with histologic subtype, pathologic T stage, and pathologic N stage. The error rate, coverage, consistency, and complexity of the decision trees were used to assess their adequacy.

Results: The trees consistently bifurcated at the first node based upon the histologic subtype. Squamous cell carcinomas were discovered to be a subpopulation for whom a molecular profile showed high accuracy and coverage in predicting differentiation. Given this result, the squamous cell carcinomas were analyzed alone. These were 15 cases of 6 moderately and 9 poorly differentiated tumors. The most consistent model that emerged from the analyses used low expression of glutathione S-transferase M1 (GSTM1) to predict poor differentiation with >90% accuracy. It was postulated that flavopiridol could be used to conjugate hydrophobic electrophiles that can otherwise cause DNA damage. Published reports show that polymorphisms leading to low activity of GSTM1 are associated with a higher risk of developing squamous cell cancers of the head and neck, of with poor survival in non-small cell lung cancer.
Conclusions: 1) Low expression of GSTM1 predicts poor differentiation in lung SCC, and can discover subpopulations with specific, predictive molecular profiles in a large, heterogeneous population. 3) This approach may be useful in the development of molecular targets for therapy in cancer patients.

1377 Similar Age-Specific Incidence Rate Patterns Imply a Biological Relationship for Ovarian Cancers
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Background: Our current pathologic classification of cancer is based on morphology, which only reflects one aspect of comparative tumor biology among neoplasms. We have used population-based age specific incidence rates, as a marker of tumor development and biology, to analyze graphically patterns among specific types of ovarian tumors.

Design: Data included 50,390 cases of ovarian cancer reported to the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute. All cases were diagnosed between 1973 and 2001. The analysis included a comparison of the age-specific incidence rates of the different morphological types plotted on a log-log scale at 5-year intervals.

Results: Rates for endometrioid carcinomas and stromal cell tumors increased until age 50, and then graphically plateau, suggesting hormonal control. The rates of serous and mucinous tumors increased until age 60, and then plateau. Granulosa cell tumors were most common around age 45. Mullerian mixed tumors and undifferentiated carcinomas occurred predominately after age 50. The rate patterns of serous papillary, serous surface papillary, and serous non-papillary carcinomas were similar. Rates for adenocarcinoma NOs and malignant epithelial tumors NOs increased steadily until age 85. The age-specific rate patterns of borderline tumors compared to corresponding invasive carcinoma subtypes were similar.

Conclusions: Age specific incidence rate patterns reflect cancer development and represent a component of tumor biology among ovarian tumors. The pattern similarity among serous tumors suggests that they represent a single biologic population and should not be divided into subtypes. Age specific rate patterns may serve as an adjunct for the biological classification of cancer.

1378 TAG-72 and MUC Protein Expression in Normal, Malignant, and Metastatic Colonic Cancer
CL Hitchcock, D Gonda, LE Hitchcock, P Wen, EJ Martin, Jr; The Ohio State University, Columbus, OH.

Background: Previous studies of monoclonal antibodies to the high molecular weight TAG-72 antigen have identified the s-Tn antigenic epitope in association with intracellular and extracellular mucin in colorectal carcinomas and in regional and distant lymph nodes. Injection of 1112-tabeled antibodies to TAG-72 and subsequent removal of antibody has resulted in significant improvement in patient survival over the following 5 years. This study was undertaken to better understand the relationship of the TAG-72 associated molecules with MUC gene product expression colorectal carcinoma.

Design: Slides were reviewed and appropriate blocks obtained from 27 stage III colorectal carcinomas. Sections selected for study included: distant margin of resection, tumor-normal colon interface, and lymph nodes with and without metastatic disease. Monoclonal antibodies include MUC-1 and MUC-6 (Novocastra Laboratories), MUC-2, MUC-4, and MUC-SAC (Zymed Laboratories), and B72.3 (Signet Laboratories). Optimal antigen specific retrieval methods and immunohistochemical staining conditions were established for each antibody using Duko Autostainer.

Results: There is apparent up-regulation of Tag-72 antigen expression in colonic epithelial cells associated with the absence of intratumoral CD31 positive microvessels. In 11/12 cases (91.7%) the absence of microvessels was noted. There was no apparent decreased expression of MUC-1 or MUC-6 compared to normal epithelial cells in the colon. The increased expression of Tag-72 was compared to normal mucosa and normal colon interface. There was no apparent decreased expression of MUC-1 or MUC-6 compared to normal epithelial cells in the colon. The increased expression of Tag-72 was compared to normal mucosa and normal colon interface.

Conclusions: TAG-72 expression in the colon alters the expression of MUC products and mucins in normal colonic epithelia cells. There is extensive heterogeneity in the expression of TAG-72 and MUC gene products within normal colonic mucosa, primary and metastatic tumors. TAG-72 and MUC-4 gene product exhibited similar staining patterns in primary and metastatic tumor.

Tumor Staining
Antibody
TAG-72
MUC-1
MUC-2
MUC-4
MUC-SAC
MUC-C
12/25 (48.0%)
22/25 (88.0%)
17/25 (68.0%)
20/25 (80.0%)
19/25 (76.0%)
20/25 (80.0%)

Staining Pattern
Extracellular & cellular
Extracellular & cellular
Extracellular & cellular
Extracellular
Extracellular
Weak extracellular

1379 Comprehensive Analysis of the Expression of the Metastasis-Associated Gene 1 (MTA1) in Neoplastic Tissue
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Background: The metastasis-associated gene 1 (MTA1) is over expressed in several human cancers and recent reports suggest that MTA1 may play a role in cancer progression either through transcription repression and/or hormone receptor interactions.

Design: In order to perform a broad survey of MTA1 expression in human tissues, we used a combination of in situ analysis of publicly available expression array data and high-density tissue microarrays (TMA). Over 90 expression array studies were queried using Oncomine (www.oncomine.org), an internet-based compendium of expression array data. TAMs created a list of over 150 different localized neoplasms including benign tumors as well as epithelial and hematopoietic malignancies were used to confirm protein expression.

Results: A comparison of both the expression array data and the results of immunohistochemical analysis of MTA on these TMAs confirmed the oncomine database findings. MTA1 expression was ubiquitously expressed both in benign and malignant tumors. MTA1 expression was found to be associated with an invasive and migratory phenotype. In 7/16 expression array studies, the tissue types compared to each other were also represented on our TMAs, and in all cases both approaches showed the same results. At the protein level, the highest levels of MTA1 expression were observed in diffuse B-cell lymphoma (mean staining intensity 3.9 on a scale of 1 to 4), basal cell carcinomas (3.7/4), and consistently in tumors with neuroendocrine differentiation such as paragangliomas (3.7/4), small cell carcinoma of the urinary bladder (3.5/4) and carcinoid tumor (3.1/4).

Conclusions: This study characterizes MTA1 expression for the first time across the entire spectrum of primary tumors, demonstrating expression in both benign and malignant neoplasm in addition to showing an association with tumors demonstrating an invasive phenotype and neuroendocrine differentiation. This study further supports the observation that MTA1 expression is associated with tissue invasion but may not be sufficient for the progression to metastatic stages.

1380 Evaluation of Microvessel Including Lymphatic Vessel Density In Endometrial Carcinoma, Carcinosarcoma and Soft Tissue Sarcoma
G Liu, M Huang, Y Fu; Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.

Background: The role of lymphatics and the presence of functional lymphangiogenesis during tumor growth and metastasis is largely unknown and under debate. It is known that carcinomas typically metastasize first to local lymph nodes through lymphatics then to distant site of the body, while the majority of soft tissue sarcomas (>90%) metastasize early to distant sites via hematogenous routes. Therefore, it is of great interest to compare macrovascular especially lymphatic vessel density between carcinomas and sarcomas. To achieve this, we used D2-40, a novel monoclonal antibody that has shown to be specific for lymphatic endothelium, and CD31 which binds to all vascular endothelium, to immunostain a series of human tumors including endometrial carcinoma, carcinosarcoma and soft tissue sarcomas.

Design: Paraffin sections of surgical specimens from 11 patients with endometrial carcinoma, 6 patients with endometrial carcinosarcoma and 5 patients with various histologies of high-grade spindle cell sarcoma were immunostained with mouse anti-human D2-40, and CD31 antibodies and the slides were assessed using an Olympus microscope. Five random images were taken from each specimen and used for statistical analysis.

Results: All tumor samples studied have intratumor CD31 positive microvessels. On average, sarcomas have the highest number of microvessels (MVD=1.355; p=0.065) compared to endometrial carcinoma (MVD=0.517) and carcinosarcoma (MVD=0.065).

Conclusions: The distribution of microvessels is diffuse in sarcomas but may be clustered in some carcinomas. Four out of five sarcomas did not have any intratumor D2-40 positive lymphatics, while all carcinomas had variable amounts of lymphatic vessels (MVD=0.225). In carcinosarcomas, the lymphatics were seen mostly within carcinomatous, instead of sarcomatous areas (MVD=0.119). In one patient who had simultaneous carcinosarcoma of the endometrium and metastatic lymph nodes, the sarcoma showed more lymphatic vessel density within the tumor was high (MVD=0.419).

Conclusions: The majority of high grade spindle cell sarcoma in this study have a high number of intratumor microvessels, but no or minimal lymphatics. The results suggest that high blood vessel density and lack of lymphatics in sarcoma may be responsible for the fact that they metastasize hematogenously early to distant sites, instead of through lymphatics to the local lymph nodes.

1381 Spectrum of KOC (K Homology Domain Containing Protein Over-Expressed in Cancer) Immunostaining among Carcinomas of Different Sites
S Evansic, GR Fanger, AE Fraire, A Khan, C Li, RK Kantis, UMass Memorial Health Care, Worcester, MA; Corixa Corporation, Seattle, WA.

Background: We have shown that KOC (K homology domain containing protein over-expressed in cancer), an oncetel RNA-binding protein, is expressed in pancreatic adenocarcinoma using a novel monoclonal antibody. KOC immunostaining has not be systematically evaluated in other carcinomas and, thus, its specificity for pancreatic cancer is unclear. The aim of this study was to assess KOC expression among carcinomas from different organs in order to establish the spectrum of staining for this new marker.

Design: Using tissue sections from paraffin-embedded blocks containing lesional and non-neoplastic tissue from 306 carcinomas (breast 26, lung 20, esophagus 14), stomach (25), colorectum (37), anus (10), pancreas (38), liver (18), salivary gland (10), cervix (9), skin (22), bladder (25), kidney (24), thyroid (24), parathyroid gland (3) were immunostained for KOC using the standard ABC technique. Staining of >10% of the tumor was considered a positive result. Any staining of non-neoplastic tissue was also noted.

Results: 127/306 (41.5%) tumors were positive for KOC (Table 1). 37/38 (97%) pancreatic carcinomas were positive for this marker, followed in frequency by carcinomas of lung and remaining gastrointestinal tract. Staining was most common in squamous cell carcinoma and adenosarcoma, whereas other types of carcinoma (e.g. parathyroid gland) were infrequently positive for KOC. All non-neoplastic tissues were negative for KOC in all cases.
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Conclusions: KOC staining is present in many carcinomas and is not a useful discriminator between carcinomas arising in different organs. However, KOC is a highly specific marker for malignancy and thus, may be useful in distinguishing between invasive carcinomas, dysplastic lesions and non-neoplastic tissues in specific situations.

1382 Determination of Evolutionary Mutation Accumulation in Neoplastic Diseases in Pancreas and Bile Duct Brushing Cytology

The molecular pathogenesis of pancreatic and biliary neoplasia involves a narrow and distinct subset of defined mutations that includes the studied mutaton acquisition could be used for distinguishing neoplastic from early and advanced cancer as well as characterize tumor aggressiveness and treatment response. Using quantitative methods applied to microdissected cell clusters selected according to morphologic features, we sought to demonstrate the feasibility and efficacy for determining the time course of mutation accumulation in pancreatobiliary cytology specimens.

Design: 40 pancreatic and 21 biliary duct brushing cytology specimens were retrieved from the cytology database. Xylene resistant markings were placed on the slide underside and coverslips removed. Clusters of normal, atypical and malignant cells were manually microdissected and DNA extracted. Mutations (allelic imbalance [LOH]) were quantitatively determined for a broad panel of 15 markers.

Results: The descending frequency of mutational content in microdissected samples of pancreatic neoplastic lesions was K-ras-2 point mutation (58%), 3p & 15q32 (35%), 5q (33%), 1p (28%), followed by the remaining molecular markers. The descending percentage of mutated cells followed essentially the same order. K-ras-2 point mutation occurred in all but 1 malignant case and contained at least two mutations of the remaining four markers. In the case of biliary cytology specimens, the mutation order was distributed with 17p13, 1p, 3p and 5p representing early mutations.

Conclusions: The pathogenicity of pancreatic and biliary neoplasia involves a unique and distinct subset of defined mutations that includes the studied mutations occurring early in tumorigenesis. K-ras-2 point mutation is most frequently acquired early and is associated with a more rapid mutation acquisition in pancreatic brushing specimens. The time course of mutation accumulation can be determined in cytology specimens and can be incorporated into the diagnostic characterization of the neoplasm.

1383 Distinguishing Fibrovascular Septa from the Extravascular Patterned Matrix of Vasculogenic Mimicry

The histogenesis of PAS+ (laminin-rich) extravascular patterned matrix in highly invasive melanomas is controversial. Molecular analyses of matrix components suggests these patterns are generated by invasive tumor cells (vasculogenic mimicry). Some observers, however, consider these patterns to represent fibrovascular septa that originate predominantly from a stromal host.

Design: Histologic sections of 234 primary uveal melanomas were stained by PAS and trichrome to identify the frequency distribution of looping patterns and any association with outcome. The circumference thickness of trichrome+ and PAS+ loops was compared by quantitative morphometry. Adjacent sections from 13 additional primary uveal melanomas that contained PAS+ loops but not thick trichrome+ loops were stained for collagen I. RQ-PCR was performed on RNA extracted from low-invasive and high invasive primary uveal melanoma cells for the expression of collagen I (COL1A2). 3D rafts composed of pure type I collagen were seeded with highly invasive or poorly invasive tumor cells and the rafts were photographed and weighed over 7 days.

Results: Trichrome+ loops were identified in 24 of 234 cases (10%) compared with PAS+ loops in 131 cases (56%). Although the detection of PAS+ loops was associated with death from metastatic melanoma (p<0.0001), there was no association between trichrome+ loops and tumor-associated mortality (p=0.5702). Trichrome+ loops were significantly thicker than PAS+-positive loops (P=0.0001). By immunohistochemistry, collagen I was detected in thin PAS+ patterns in 8/13 cases and within tumor cell cytoplasm. RQ-PCR revealed a 97% increase in expression of collagen I by highly invasive primary melanoma cells compared with poorly invasive cells. Floating rafts composed of type I collagen were degraded by highly invasive melanoma cells but not by poorly invasive tumor cells.

Conclusions: Fibrovascular septa are rare and prognostically insignificant in uveal melanomas because highly invasive tumor cells degrade collagen I. By contrast, the extravascular patterned matrix is associated with increased mortality. Limited distribution of collagen I in the extravascular patterned matrix may result from its synthesis and degradation by tumor cells, independent of a host stromal response. Supported by NIH grant EY10457

1384 Intra-Tumor Depletion of CD4+ Cells Unmasks Tumor Immunogenicity Leading to Rejection of Established Tumor

The histogenesis of PAS+ (laminin-rich) extravascular patterned matrix may result from its synthesis and degradation by tumor cells, independent of a host stromal response. Supported by NIH grant EY10457

Background: The frequent observation of T cell infiltration into cancer tissues indicates that immune recognition of cancer occurs. However, it is exceptional for such tumor infiltrating T cells to induce the spontaneous rejection of established tumors. Many studies present the possibility of a local environment at the tumor site may play critical roles for preventing the immunological destruction of antigenic tumors. The Foxp3 expressing CD4+CD25+ T cell subset severely limit the efficacy of vaccine-induced antitumor immune responses through inducing the CD8+ T cells. We sought to determine whether abrogating the effects of CD4+CD25+ T cells at the tumor site could be an effective approach to enhance anti-tumor immunity, leading to rapid rejection of well-established tumors.

Design: Human breast cancer samples (26 DCIS and 20 infiltrating ductal carcinomas) were stained with anti-CD4, CD8, CD25 and FOXP3 antibodies. The Ag104 fibrosarcoma and Ag104 cell line expressing murine H-2Ld were injected to mice. CD4+ and CD25+ cells were isolated from the tumor murine and the regulation of CD8+ cells by CD4+CD25+ cells was studied. Intra-tumoral injection of anti-CD4 and CD25 antibodies was carried out. We analyzed the changes of tumor sizes, intra-tumor CD8+ number, inflammatory and anti-inflammatory cytokine expression levels andfox3 gene expression.

Results: The vast majority of lymphocytes are CD8+ T cells in breast DCIS, however, the CD4+ T cell population increased dramatically in infiltrating ductal carcinomas. Similar phenomena were demonstrated in murine model. CD4+CD25+ T cells suppressed the proliferation and interferon-γ production of CD8+ T cells at the local tumor site. Blockade of the effects of IL-10 and TGF-β could partially reverse the suppression induced by the CD4+ cells. Local depletion of CD4+ cells leads to the eradication of well-established tumors and to the development of long-term anti-tumor memory.

Conclusions: There was a selective accumulation of CD4+CD25+ T cells inside malignant tumors. CD4+CD25+ T cells primarily mediated a suppressive environment inside the tumor and abrogated the effect of CD8+ T cells. Intra-tumoral depletion of these regulatory T cells unmasks the immunogenicity of tumor and reverses CTL tolerance leading to the rapid rejection of well-established tumors. Regulatory cells could be one important local factor to suppress immune responses against a strong tumor antigen leading to progressive growth of cancer in the immune competent hosts.

1385 Overexpression of RNase L Inhibits Tumor Growth in Fibrosarcoma in Nude Mice

The histogenesis of PAS+ (laminin-rich) extravascular patterned matrix in highly invasive melanomas is controversial. Molecular analyses of matrix components suggests these patterns are generated by invasive tumor cells (vasculogenic mimicry). Some observers, however, consider these patterns to represent fibrovascular septa that originate predominantly from a stromal host.

Design: To directly measure the effect of RNase L on tumor growth in the absence of other IFN-induced proteins, human RNase L (DNase) was stably expressed in P-57 cells, an aggressive mouse fibrosarcoma cell line. Several clonal cell lines were isolated in which overexpression of RNase L was 20-30 fold of the endogenous level. Groups of three nude mice were injected subcutaneously with either the human RNase L overexpressing clones (PL-RNase L) or control transfected with an empty vector (PL-vector). Tumor formation by these two cell lines was monitored by measuring tumor volume. The University of Chicago Hospitals, Chicago, IL.

Results: In the RNase L+ group, tumor formation was significantly delayed and the tumors grew much slower compared to the control group. Morphologically, the RNase L+ tumors cells changed from spindle cells with a herringbone pattern to more polygonal cells, demonstrated more discosclerosis, and showed increased areas of tumor necrosis. Interestingly, after 5 weeks, the growth of RNase L+ tumors started to accelerate. Eventually tumors in both groups reached the same size, corresponding to the fact that the expression of RNase L was completely shut down in RNase L+ group.

Background: RNase L is one of the key enzymes involved in interferon signal transduction pathways. It mediates the antiviral and anti-proliferative functions of interferon. RNase L is also found to exert proapoptotic activity independent of interferon. However, the exact function of RNase L in tumorigenesis is unknown. This study was designed to study the role of RNase L in tumor growth using a nude mice model.

Design: To directly measure the effect of RNase L on tumor growth in the absence of other IFN-induced proteins, human RNase L (DNase) was stably expressed in P-57 cells, an aggressive mouse fibrosarcoma cell line. Several clonal cell lines were isolated in which overexpression of RNase L was 20-30 fold of the endogenous level. Groups of three nude mice were injected subcutaneously with either the human RNase L overexpressing clones (PL-RNase L) or control transfected with an empty vector (PL-vector). Tumor formation by these two cell lines was monitored by measuring tumor volume.

Results: In the RNase L+ group, tumor formation was significantly delayed and the tumors grew much slower compared to the control group. Morphologically, the RNase L+ tumors cells changed from spindle cells with a herringbone pattern to more polygonal cells, demonstrated more discosclerosis, and showed increased areas of tumor necrosis. Interestingly, after 5 weeks, the growth of RNase L+ tumors started to accelerate. Eventually tumors in both groups reached the same size, corresponding to the fact that the expression of RNase L was completely shut down in RNase L+ group.

Supported by NIH grant EY10457
Conclusions: Overexpression of RNase L significantly inhibits the tumor growth, suggesting that RNase L plays a critical role in growth regulation of fibrosarcoma in nude mice.

1386 Elevated Expression of BP1 in Both Invasive and Metastatic Inflammatory Breast Cancer

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Background: BP1, a homeobox gene, is expressed in an increasing percentage of hyperplastic, in situ, and invasive breast lesions, in contrast to its sparse detectability in normal human breast tissues. This study investigates the immunoeexpression of BP1 in inflammatory breast cancer (IBC), a relatively rare but very aggressive form of breast cancer characterized by extensive lympho-vascular invasion.

Design: Paraffin-embedded tissue sections from 40 cases of IBC, seven with paired normal breast tissues, were assessed immunohistochemically (IHC) and immunostaining for breast cancer was scored in a panel of markers specific to epithelial cells, blood vessels, and lymphatic channels. Results: BP1 immunoreactivity was identified in all cases of IBC with intensities ranging from focal to diffuse and strong. Adjacent benign breast tissues were immunoreactive in a minority of cases, similar to our previous results. Immunoreactivity of metastatic tumors was equal to or greater than the staining reactivity present in the primary breast carcinoma. Carcinoma within lymphatic channels was uniformly positive for BP1 immunoreactivity. The percentage and distribution of immunoreactivity in the cohort of IBC cases were greater than a comparison cohort of non-IBC ductal carcinomas.

Conclusions: BP1 expression was identified uniformly in IBC and its metastases. Current and previous studies suggest that BP1 may act as an oncogene promoting tumor progression and metastasis.

1387 EBV Infection of NOD/SCID Mice Reconstituted with Human Hematopoietic CD34+ Cells: A Potential In Vivo Model for EBV Induced Lymphoproliferative Disorder (LPD) in Immunodeficient Patients

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Background: EBV-induced LPD is a serious complication of immune deficiency. Inadequate T-cell suppression allows the outgrowth of transformed cells resulting in B-cell lymphomas. The mechanism by which EBV transforms B-cells is not well understood. An in vivo model that recapitulates EBV infection and its association with B-cell lymphomas is currently lacking. Experimental EBV infection of NOD/SCID mice reconstituted with human CD34+ stem cells could serve as such a model.

Design: NOD/SCID mice engrafted with human CD34+ cells and reconstituted mainly with B-cell lymphomas. The oncogenic machinery of these lymphomas may provide avenues for therapeutic interventions.

Conclusions: NOD/SCID mice engrafted with human CD34+ cells and reconstituted mainly with B-cell lymphomas. The mechanism by which EBV transforms B-cells is not well understood. An in vivo model that recapitulates EBV infection and its association with B-cell lymphomas is currently lacking. Experimental EBV infection of NOD/SCID mice can produce lymphomas with features similar to human B-cell lymphomas. This model may provide valuable insights into the pathogenesis and potential therapeutic targets for lymphomas associated with EBV infection.

1388 Colonic Adenocarcinomas Show Increased Ratio of Saturated to Unsaturated Fatty Acids When Compared to Normal Colonic Mucosa

D Rabbeja, P Kapur, MP Hoang, MJ Bennett. University of Texas Southwestern Medical Center, Dallas, TX.

Background: Mammalian fatty acid synthase (FASAE) overexpression has been shown in a number of human malignancies, including colonic adenocarcinoma. Since FASE synthesizes only saturated fatty acids, and since humans do not have the enzymatic machinery to convert these saturated fatty acids to polyunsaturated fatty acids, we hypothesize that cancer cells have a greater proportion of long-chain saturated fatty acids.

Design: Fresh tissue samples from 13 colonic adenocarcinomas and adjacent normal colonic mucosa were obtained from surgical specimens. The samples were homogenized followed by one-step lipid extraction and methyl esterification. Palmitoleic, oleic, and linoleic acids were then measured by gas chromatography-mass spectrometry in selective ion mode. Statistical analyses were performed on Microsoft Excel spreadsheet using a tailed Student’s t-test.

Results: There was a relative abundance of stearic acid in colon carcinoma as compared to normal colonic mucosa, as shown in the Table.

Conclusions: Our study shows unequivocal increase in saturated C18 fatty acid (stearic acid) compared to adjacent normal colonic mucosa. This may create significant alterations in physical and biological properties of cancer cells, providing avenues for therapeutic interventions.

1389 A Distinctive Association of Activated mTOR Pathway in Human Epithelial Tumors: Akt and 4EBP1 as Targets of Cell Signalling

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Background: Activation of the PI3-K/Akt/mTOR signal transduction pathway contributes to the development and progression of tumors by prevention of apoptosis and deregulation of the cell cycle. Dissecting the molecular events of this pathway may provide instrumental knowledge to understand a different potential behaviour in tumors, mechanisms of activation of this signalling cascade and implications for the development of therapeutic PI3K/Akt/mTOR inhibitors.

Design: We have analysed 247 paraffin-embedded human malignant epithelial tumors: 112 ovary, 56 breast, 25 gastric, 25 colon, 19 prostate and 10 pancreas, with a complete immunohistochemistry profile including multiple phosphorylated (p) downstream proteins: pAkt, p4EBP1, p70S6K and pS6. The levels of expression were evaluated as percentage and intensity of stained tumor cells (Hiscore).

Results: Globally, there was a significant correlation between the levels of pAkt, p4EBP1, p70S6K and pS6 (Pearson’s r, p=0.005). Interestingly, high pAkt expression was consistently observed in all tumor types whereas the downstream effectors presented higher activation in pancreatic, breast and prostate than in gastric and colon carcinomas (table, Pearson Chi square, p=0.005). Activated mTOR downstream effectors were mainly detected in poorly differentiated tumors.

Conclusions: Detection of p proteins of the Akt/mTOR pathway is feasible in a variety of tumor types. Our findings suggest that level of in vivo activation of Akt is significant in a variety tumor types but activation of mTOR downstream proteins could be higher in different tumor types by alternative mechanisms: both Akt and 4EBP1 can be expressed concomitantly or independently in tumors and be associated with different signalling pathways that result in proliferation, apoptosis and cell growth. The oncogenic alterations that mediate and drive these labyrinthine cellular pathways have to be determined.

1390 A Mouse Model of Gastrointestinal Stromal Tumor

BP Rubin, ML Comstock, JP Scott-Browne, MR Tanas. University of Washington Medical Center, Seattle, WA.

Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Most GISTs harbor activating mutations in Kit, a receptor tyrosine kinase (RTK). Rare families have germline activating mutations in Kit that are inherited, allowing them to germinate Kit mutations and family members with these mutations develop interstitial cell of Cajal (ICC) proliferations and GISTs. We have constructed a mouse model harboring a germline activating Kit mutation, which mimics the human familial GIST syndrome.

Design: We have used a genetic knock-in strategy to replace one normal Kit allele with a mutant Kit allele that harbors a K641E mutation. This mutant allele is analogous to a mutation seen in a single human familial GIST syndrome family. Mice harboring
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1391 DNA Methylation Regulates Galectin-3 Expression in Various Cell Lines and in Pituitary Tumors
KH Ruebel, L Jin, BW Scheithauer, K Korsak, RV Lloyd. Mayo Clinic, Rochester, MN; St. Michaels Hospital, Toronto, ON, Canada.
Background: The LgalS3 gene is expressed in many tissues and increased expression has been associated with some malignant tumors. Galectin-3 (Gal-3) protein was recently observed in subsets of pituitary tumors, and there was increased expression in pituitary carcinomas. The mechanisms regulating Gal-3 expression are unknown. Design: We hypothesized that unmethylated region of the LgalS3 gene by methylation specific PCR (MS-PCR) and DNA sequencing in various tumor cell lines including breast carcinomas (n=3), thyroid carcinomas (n=3), a pituitary adenoma and HeLa cell lines. Twenty-three pituitary tumors were also examined. Gal-3 protein expression was also analyzed by Western blotting.
Results: In most cell lines, the LGAL3 gene was unmethylated and there was expression of Gal-3 protein. The SKBR3 breast carcinoma cell line was methylated and did not express Gal-3. Treatment with 5'-azacytidine resulted in expression of Gal-3 protein. The SKBR3 breast carcinoma cell line was methylated and did not express Gal-3. Treatment with 5'-azacytidine resulted in expression of Gal-3 protein. The SKBR3 breast carcinoma cell line was methylated and did not express Gal-3. Treatment with 5'-azacytidine resulted in expression of Gal-3 protein.
Conclusions: Epigenetic inactivation of LGALS3 is an important mechanism regulating Gal-3 expression. Analysis of Gal-3 methylation may be a useful diagnostic marker.

1392 The Ataxia-telangiectasia Gene Products Require for Genomic Stability Following Labile Ferric Iron Exposure
RE Shackleford, RP Manucat, S Wang, M Lowery-Norberg, A Chen. Louisiana State University System, Shreveport, LA; Iowa Cancer Research Foundation, Urbandale, IA.
Background: The Ataxia-telangiectasia (A-T) is a rare autosomal recessive disorder characterized by immune dysfunction, progressive cerebellar ataxia, and an 1,000X elevated cancer incidence. The gene mutated in A-T, ATM, has been cloned and exhibits several functions. A cytoplasmic/secretory form of the clusterin protein that has calcium responsiveness. Promoter analysis identified several key regulatory sequences of the main promoter, including a monomeric nuclear receptor response element (NRE) and two cAMP responsive elements (CREs). CRE binding protein (CREB) interacts with CREs in neurons and steroidogenic factor 1 (SF-1) binds to NRE in pituitary gonadotropes. In the CNS, the transcription factors that bind to the NRE needs to be clarified.
Results: We show that modest increases in labile ferric iron (a 100nM increase) significantly lowered A-T, but not normal cell viability. Labile iron also increased A-T, but not normal cell dIdNA breaks at low concentrations. These effects were absent in A-T cells expressing functional ATM protein. Iron chelators also increased A-T, but not normal cell genomic stability and viability, with and without exogenous oxidative stress. Last, interperitoneal injection of Atm-deficient mice with the iron chelator deferl salferal increased Atm-deficient mouse weight.
Conclusions: ATM is required for the maintenance of cell viability and genomic stability in the face of low labile ferric iron levels. Additionally iron chelators increased A-T cell genomic stability and viability, and Atm-deficient mouse weights. Iron chelators may therefore prove useful in the treatment of A-T. Also, since iron plays a central role in several diseases, such as hemochromatosis and Parkinson's disease, our data indicates that ATM function may prove important in these disease processes.

1393 Lens-Like Expression of Crystallins in the Corneas of Cats with Mucopolysaccharidoses I and VI
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Background: Lysosomal storage disorders represent a group of over 45 distinct genetic illnesses, with an estimated incidence of 1/7,000 live human births. Lysosomes catabolize cellular and extracellular macromolecules and provide substrate for resynthesis of cellular and extracellular molecules. A common biochemical feature of lysosomal storage is the accumulation of undegraded or partially degraded substrates within the lysosomes leading to cell dysfunction. A phenotypic characteristic of humans and animals affected with mucopolysaccharidosis (MPS) 1 (Hurler disease), MPS IV-A (Morquio A disease), MPS VI (Maroteau-Lamy disease), and MPS VII (Sly disease) is cloudy cornea. Current morphologic explanations for this phenotype include the presence of enlarged secondary lysosomes packed with storage material, and the disorganization of collagen in the corneal matrix; however, the molecular basis remains unknown.
Design: In this study we utilized differential display proteomics with “fused and super-fused” images using the software Delta 2D (Decodon.com) to compare corneas of cats affected with MPS I and VI or normal age/matched controls.
Results: A total of 2729 spots were detected and 53 spots were found specifically upregulated (over three-fold). The top twelve upregulated spots were punched out of the gels, digested with trypsin and fed into a micro HPLC tandem mass spectrometer (40 LS-MS-ESI DECA Thermal-Hyperion). Identification of proteins was performed with the software Sequest and it showed a family of proteins not previously identified in the cornea of humans or animals; the lens proteins crystallin alpha, beta and gamma.
Conclusions: We hypothesize that cell trafficking dysregulation caused by the truncated gene product of the enzymes alpha-L-iduronidase and N-acetylgalactosaminase 4-sulfatase per-se or the accumulation of dermatan sulfate and heparan sulfate in MPS I and dermatan sulfate and chondroitin sulfate in MPS VI as well as a decreased activity of the enzymes 4-sulfatase and alpha-L-iduronidase. We have developed a mouse GIST model in which 100% of the mice develop tumors and express Gal-3. The development of a mouse GIST model, which also express Gal-3, may aid in the understanding of the pathogenesis of GISTs as well as serving as an excellent pre-clinical model to test the efficacy of therapies which target Kit.

1394 Dynamic Duo Orchestrates the Double-Edged Sword
EX Wei, M Sasaki, VL Davidson, TM Dawson. Louisiana State University System, Shreveport, LA; Johns Hopkins University, Baltimore, MD.
Background: Nitric oxide (NO) plays a critical role in neuronal signaling, cerebral ischemia, neuronal injury and neurodegenerative disorders. Neuronal nuclear oxynitrite synthase (nNOS) accounts for the majority of actions of NO in the cerebral vascular system. nNOS mRNA and protein is significantly upregulated by calcium influx under ischemic or injurious conditions. Multiple promoters control nNOS transcription. The major promoter resides at the proximal promoter and confers calcium responsiveness. Promoter analysis identified several key regulatory sequences of the main promoter, including a monomeric nuclear receptor response element (NRE) and two cAMP responsive elements (CREs). CRE binding protein (CREB) interacts with CREs in neurons and steroidogenic factor 1 (SF-1) binds to NRE in pituitary gonadotropes. In the CNS, the transcription factors that bind to the NRE needs to be clarified.
Results: Rat embryonic cerebral cortical neurones were cultivated and various nNOS reporter constructs and overexpression plasmids were transfected. The reporter activities were analyzed. Gel shift assays (GSA) were performed using neuronal nuclear extracts.
Conclusions: Deletion and mutation of the NRE dramatically diminished nNOS calcium inducibility. Although only exogenous SF-1 significantly increased nNOS promoter activity, the nature of the protein was not definitively determined, as antibodies against known monomeric nuclear receptors (NRs), including SF-1, were not able to block transactivation of the nNOS promoter. nNOS mRNA and protein were not detectable in the nerve growth factor (NGF) treated neuronal cell line, although NGF induced CREB expression. It is likely to be a SF-1-like NR family member. It cooperatively interacts with CREB and the NR coactivators SRC-1 and CBP, and regulates nNOS transcription by calcium influx. COUP-TFI, a transrepressor, is a dimeric NR essential for neuron development and capable of binding to monomeric and dimeric NRs. COUP-TFI binds to the same nNOS NRE in GSA, but with less affinity and forms its own distinct band. It recruits corepressor SMRT and suppresses nNOS transcription. It dose-dependently competes with SF-1-like protein and vice versa.

1395 Role of p53, IGFR and EGFR in Clusterin Expression in Rectal Cancers
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Background: Clusterin is a ubiquitous cellular glycoprotein that has been implicated in a number of pathways of carcinogenesis, as well as in cellular systems associated with cell injury leading ultimately to cell death. Studies in different models and organ systems have recently recognized two different isoforms with dramatically different functions. A cytoplasmic/secretory form of the clusterin protein that has cytotoxic effects and is associated with tumor progression. Additionally, a nuclear form has recently been identified whose activation is related to cell death processes and turnover of dying cells. Studies performed on cell culture models in our laboratory have implicated p53 and IGF pathways in regulating clusterin formation and function and excluded EGF pathway as an important modulator of clusterin
expression. To further explore this hypothesis we investigated immunohistochemical staining for nuclear and cytoplasmic clusterin expression in a cohort of rectal cancers and investigated its co-expression with p53, EGFR and IGF.

**Design:** Representative tissue blocks from 37 rectal cancer resections retrieved from our surgical pathology department archive were used. These neoplasms were immunostained using commercially available antibodies for nucleotides and secreted forms of clusterin, p53, phosphorylated EGFR and non-phosphorylated IGF. Each slide was scored independently and results were expressed in terms of percentage of tumor cells staining ≥ intensity.

**Results:** Mean score and range for each stain was as follows: nuclear clusterin 10 [0-270]; cytoplasmic clusterin 30 [0-240]; p53 210 [10-270]; IGF 100 [0-270]; EGFR-Phos 120 [0-270]. Using a Spearman R test a significant negative correlation was identified between expression of nuclear clusterin and IGF as well as nuclear clusterin and p53 staining. A positive correlation was identified between cytoplasmic clusterin and cytoplasmic EGFR-Pho. No correlation was found between nuclear and cytoplasmic clusterin.

**Conclusions:** These findings corroborate our previously reported cell culture experiments of an association between nuclear clusterin and p53 status in tumor cells. These results support the concept of a pivotal role of IGF in the modulation of nuclear clusterin expression. The data also suggest that in rectal cancer EGFR activation may signal secretory clusterin expression.

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**1396 Involvement of CD5 and CD10 in the Development of Choroid Plexus Papillary Neoplasms**

**E Yu, SH Son, EK Choi, H Lee, J Choi. University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.**

**Background:** Choroid plexus papillomas (CPPs) are benign, relatively uncommon neoplasms that occur especially in children. The epithelial nature of the normal choroid plexus and choroid plexus neoplasms has been confirmed by previous studies demonstrating positive expression of epithelial markers such as cytokeratins and EMA. CD5 and CD10 are membrane glycoproteins used extensively in working up lymphoproliferative disorders, but have also been shown to be expressed in a variety of normal epithelium and carcinomas. However, the expression of CD5 and CD10 in the choroid plexus papillary neoplasms have not been studied.

**Design:** 19 cases including 8 CPPs and 11 normal choroid plexus were retrieved from the laboratory database. Immunostaining with antibodies to CD5 and CD10 was performed on paraffin-embedded tissue. Immunostains were performed on an automated immunostainer with appropriate positive and negative controls.

**Results:** CD5 immunoreactivity was demonstrated in 7/8 (88%) of choroid plexus papillomas and in only 1/11 (9%) of normal choroid plexus.

**Conclusions:** Our results showed that CD5 and CD10 is expressed in choroid plexus papillomas, but not in normal choroid plexus. The expression of CD5 and CD10 may be involved in the pathogenesis of choroid plexus neoplasm and can also be useful in separating normal choroid plexus from choroid plexus papillomas, especially when very limited material is present in the brain biopsy.

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**1397 Effects of Retinoic Acid on Promyelocytic Leukemia Protein-Induced Growth Suppression and Cell Death in Solid Tumor Cells**

**E. Je, SH Son, EK Choi, H Lee, J Choi. University of Ulsan College of Medicine Asan Medical Center, Seoul, Korea.**

**Background:** The promyelocytic leukemia protein (PML), involved in the pathogenesis of acute promyelocytic leukemia (APL), is a co-activator in p53 tumor suppressive functions. The ability of the PML to inhibit growth and to induce cell death of solid tumor cells, however, has not been determined. As a therapeutic agent in APL, retinoic acid (RA) displays antiproliferative effects in solid tumor cells of the breast, the prostate and the lung by promoting apoptosis.

**Design:** To evaluate the tumor suppressor ability of the PML in solid tumors, we first assayed the antiproliferative activities of the PML by using replication-deficient recombinant PML adenovirus (Ad-PML) and compared them with those of p53 in four liver cancer cell lines. Next, we examined whether RA has synergistic effects on the PML-induced tumor cell suppression or not.

**Results:** Following infection of cells with Ad-PML, in vitro growth curve analysis showed that the overexpressed PML initially induced a substantial G1 cell cycle arrest and triggered massive cell death in all tested cell lines, irrespective of their p53 status. The PML-induced cell death decreased by about 30% in the presence of a broad caspase inhibitor, zVAD. The cell death effect of the PML was higher than that induced by p53 over a longer period of time. As with p53, the overexpression of the PML was closely related to up-regulation of p21 and the decrease of cyclin D1. Unexpectedly, RA antagonized rather than enhanced the PML-triggered cell death. RA enhanced the expression of adenovirus-CMV-promoted PML at both transcription and protein levels within 12 h after the treatment. However, the PML was significantly degraded in the presence of RA at days 3-5 post-infection. The PML degradation was also observed in SK-BR3 breast cancer cells treated with RA. RA treatment induced retinoic acid receptor (RAR) expression. Addition of LLL1, a proteasome inhibitor, blocked RA-induced decreases in the PML and RAR proteins.

**Conclusions:** Our findings strongly support the hypothesis that the PML acts as a strong independent cell death inducer and that RA conversely abolishes the therapeutic effects of the PML through proapoptotic degradation of the protein.

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**1398 Expression of Transforming Growth Factor beta 1 and Its Receptor, Smad and JNK in Colorectal Adenocarcinomas**

**G Yue, M Tsung, YL Liu, JF Silverman. Allegheny General Hospital, Pittsburgh, PA.**

**Background:** Colorectal adenocarcinoma is one of the most common cancers and leading cause of death in United States. Transforming growth factor beta 1 (TGFb1), a potent inhibitor of cell growth, activates Smad and JNK proteins by binding its receptor. Smad and JNK are nuclear transcriptional activators regulating gene transcription. The resulting repression of c-myc and induction of cyclin-dependent kinase inhibitors leads to G1 phase cell cycle arrest. Previous studies indicated that decreased expression of Smad and JNK is associated with poor prognosis in a number of carcinomas. However, the correlation of TGFb1 and its signal transduction’s expression and prognosis in the colorectal adenocarcinoma has not been investigated.

**Background:** A total of 20 cases of primary and metastatic colorectal adenocarcinomas were retrieved from the hospital data base. In the primary colorectal adenocarcinoma without lymph node and distant metastasis, there are five each of poorly and well differentiated adenocarcinomas. Five cases of colorectal adenocarcinoma with positive lymph nodes and five metastatic colonic adenocarcinoma with liver metastases were selected. Immunostaining for TGFb1, TGFb1 receptor, Smad2, and JNK were performed on an automated immunostainer with appropriate positive and negative controls. The intensity of immunostain was measured and classified into four scale: 0:score 0; weak (score 1); strong (score 3); and between (score 2) by two observers. Statistical analysis was performed with Chi-Square test.

**Results:** TGFb1, TGFb1 receptor, Smad, and JNK were observed in all adenocarcinomas in this study. The immunostaining patterns are as follow. TGFb1 and TGFb1 receptor show membrane and cytoplasmic pattern. Smad2 shows nuclear pattern. JNK shows nuclear and cytoplasmic pattern. There is no statistic difference in the expressions of TGFb1, TGFb1 receptor, Smad and JNK in the primary and metastatic colon adenocarcinomas.

**Conclusions:** Our results indicate that the expression of TGFb1, TGFb1 receptor, Smad, and JNK can not be used as a prognostic factor for colorectal adenocarcinomas and support the hypothesis that the aggressive behaviors of colorectal carcinomas may be associated with the mutations rather than the decreased expression of TGFb1 and it’s signal transduction system.

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**1399 Induction by Selenium of Apoptosis in Endometrial Cancer Cells Is Coupled with an Increase in the Level of Active Caspase-3**

**P Zhang, C Zhang, CJ Sung, F Liu, WD Lawrence. Women & Infants Hospital, Brown Medical School, Providence, RI.**

**Background:** Selenium (SE), a constituent of antioxidant enzymes, has been proposed as a chemopreventive and chemotherapeutic agent for prostate and several other cancers; however, no information is available concerning its effect in endometrial carcinoma. Many chemotherapeutic agents inhibit cancer growth by inducing apoptosis. Caspase-3, a cellular protease, plays a major role in apoptosis. It is expressed in cells as an inactive 32 kDa precursor which, during apoptosis, forms an active 17 kDa peptide; the latter breaks down into an active 15 kDa subunit. The aim of this study is to investigate the effects of SE on cell growth, apoptosis, and the roles of different forms of caspase-3 in cultured endometrial cancer cells (EC).

**Design:** EC (American Type Culture Collection, Manassas, VA) was incubated with different concentrations of sodium selenite (0 µM, 2 µM, 4 µM, and 6 µM) for 48 hours since studies have shown that 5 µM SE treatment induces apoptosis in prostate cancer cells, but not in normal prostate cells. Cell cultures were examined under a Nikon inverted microscope and photographed with an attached Roper digital camera. MTS/MSM Cell Titer AQ Assay (Promega) was performed to determine cell viability. Apoptosis was evaluated using gel electrophoresis to detect DNA laddering. Western blotting was used to measure the levels of different forms of caspase-3.

**Results:** Direct microscopic examination revealed obvious cell death of EC cells after SE treatment. The cell viability assay showed that after a slight increase of viable cells with 2 µM SE treatment, cell viability decreased by 54% and 77% with 4 µM and 6 µM SE treatment, respectively. Apoptosis occurred with 2 µM SE treatment and increased as the concentration of SE rose. The 17 kDa subunit of caspase-3 was not detectable without SE treatment, although it became detectable after treatment with 2 µM SE, and the level increased 10-fold after 4 µM SE treatment. The 20 kDa peptide and the 11 kDa subunit only increased slightly with 2 µM or higher concentrations of SE treatment. The level of 32 kDa precursor seemed unchanged after SE treatment.

**Conclusions:** To our knowledge, this is the first study showing an anti-cancer effect of SE in EC, associated with induction of apoptosis. The concurrent increase of an active, 17 kDa subunit, but not the other forms of caspase-3, suggests that the 17 kDa subunit is more important in the caspase-3 pathway of the SE-induced apoptosis in EC.