necrosis, and adjacent thyroid pathology were assessed. IHC for CK19 and HBME1 was performed. Microdissection, PCR, and sequencing of exon 15 of the BRAF gene were also completed.
Results: Ages ranged from 14-77 (median 59) and there were 11 women and 1 man. All tumors were unifocal and half had a predominantly papillary architecture (>50% of the tumor). Microfollicular and solid growth patterns were also occasionally noted. Papillary fronds were lined by a single layer of cuboidal to columnar cells with oncocytic cytoplasm and prominent hobnailing. Nuclei were most often centrally located, although two tumors had uniformly apical nuclei. Chromatin was fine and dispersed and small nucleoli were present with varied prominence. Mitotic activity was rare and all but 2 cases had minimal to mild nuclear atypia. Although rare nuclear features of PTC were seen in 3 cases, no cases, by definition had sufficient changes to warrant a diagnosis of PTC. All tumors were encapsulated. Extracapsular extension (1/12) and vascular invasion (6/12) were sometimes noted. Calcifications (3/12) and definitive psammoma bodies (1/12) were rare. Adjacent thyroid pathology included nodular hyperplasia (4/12), mild lymphocytic thyroiditis (1/12), and one minimally invasive follicular carcinoma. No cases were immunoreactive with antibodies to HBME1 and only 1 of 9 was immunoreactive with antibodies to CK19. No BRAF point mutations were identified (0/9).
Conclusions: PONs are histologically, immunohistochemically, and molecularly distinct from PTC and appear to be most related to follicular neoplasms. If the diagnostic criteria for follicular neoplasms were used, more than half of the tumors would be considered minimally invasive or angioinvasive carcinomas.

507 PPARδ Is Over Expressed in Thyroid Tumors and Regulates Proliferation in Primary Thyroid Cells

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Background: Peroxisome proliferator-activated receptors (PPARs) are ligand-regulated transcription factors that have been implicated in lipid metabolism and the pathogenesis of thyroid tumors. Here, we determine that PPARδ is over expressed in thyroid tumors and regulates proliferation in thyroid cells.
Design: Thyroid tissue microarrays were constructed with 18 follicular adenomas, 20 follicular carcinomas, 37 papillary carcinomas, 9 Hurthle cell adenomas, 9 Hurthle cell carcinomas, 10 poorly differentiated/anaplastic carcinomas and 76 normal thyroid tissues (ATA-27, Beecher Instruments). Immunohistochemistry was performed with microwave antigen retrieval and EnVision (non-biotin) detection (DAKO) using antibodies against PPARδ (H-74, Santa Cruz) and Ki67 (Ki-S5, DAKO). Expression levels were quantitated with the Automated Cellular Imaging System (Chromovision) that recorded positive nuclear and cytoplasmic staining as a numerical score between 0 and 225 normalized to an area of 1 um^2. Brown positive staining was differentiated from blue negative counterer staining. *p* values were calculated using the Student's *t* and Spearman rank correlation tests. Immunoblots were performed with chemi-luminesence detection. PPARδ was over expressed by electroporation and knocked down by siRNA in primary human thyroid cells. Thyroid cell proliferation was determined by the incorporation of BrdU.
Results: The expression of native PPARδ was elevated 3- to 5-fold in follicular adenomas (208.44, $p<0.0001$), follicular carcinomas (221.63, $p<0.0001$), papillary carcinomas (394.11, $p<0.0001$), Hurthle cell adenomas (352.67, $p<0.0001$), Hurthle cell carcinomas (293.89, $p<0.0001$) and poorly differentiated/anaplastic carcinomas (438.60, $p<0.0001$) compared to normal thyroid tissues (75.19). Mean levels of PPARδ correlated directly with those of Ki67 (R=0.8571; $p=0.02381$) in the thyroid tumors. PPARδ ligand induced proliferation in primary thyroid cells. Engineered over expression of PPARδ increased BrdU incorporation 55%, phospho-Rb 200% and cyclin E1 900% in just 2 days. Knockdown of PPARδ by siRNA inhibited proliferation 45%.
Conclusions: Our experiments demonstrate that PPARδ is over expressed in many benign and malignant thyroid tumors. PPARδ appears to regulate proliferation in thyroid cells by modulating cyclin E1. These findings suggest that that PPARδ coordinates cell proliferation with lipid metabolism in a physiologic process that is deregulated during thyroid tumorigenesis.

Gastrointestinal

508 Paget Cells in the Esophagus: Assessment of Their Histopathologic Features and Near-Universal Association with Underlying Adenocarcinoma

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Background: Esophageal Paget disease (PD) – intraepithelial growth of neoplastic cells with glandular differentiation – has only rarely been reported. We recently encountered 3 endoscopic biopsies containing Paget cells in association with adenocarcinomas of Barrett esophagus (BE) or the esopahgogastric junction. The aim of this study was to evaluate the prevalence of primary and secondary esophageal PD and its histochemical and immunohistochemical profile.
Design: To search for primary esophageal PD, we reviewed the computerized database at our institution from 1994-2007. For secondary PD, we studied 108 adenocarcinomas (81 EMRs + 27 esophagectomies) and 72 BE with high grade dysplasia (47 EMRs + 25 esophagectomies). Cases with Paget cells (including index biopsies) were subjected to histochemistry for PAS-D and mucicarmine, and immunohistochemistry for CK7, CK20, p53, and E-cadherin.
Results: There were no cases of primary esophageal PD over a 13 year span. In contrast, Paget cells were present in squamous epithelium overlying 5/108 esopahgeal/EG junction adenocarcinomas, yielding a 4.9% prevalence of secondary PD. None (0%) of 72 BE with high grade dysplasia ("in situ" disease) had Paget cells ($p=0.16$). Among the 8 patients with Paget cells (including the 3 index biopsies) there were no differences in gender ($p=0.58$) or age ($p=0.78$) as compared to 103 adenocarcinomas without Paget cells. Morphologically, all cases with Paget cells contained at least a component of diffuse, poorly differentiated carcinoma (one was a signet ring cell carcinoma), and Paget cells involved only squamous epithelium directly above the poorly differentiated foci. Their staining profile was as follows (one case unavailable): PAS-D+ (7/7, 100%), mucicarmine+ (6/7, 86%), CK7+ (7/7, 100%), CK20+ (5/7, 71%), p53 overexpression (3/7, 43%), and E-cadherin loss (complete in 1 and faint in 3, 57%). A control group of 19 adenocarcinomas without Paget cells were also stained for E-cadherin; only 1 (5%) showed faint expression and none had complete loss ($p=0.01$).
Conclusions: Unlike mammary, vulvar, and perianal PD, esophageal Paget cells are almost universally associated with underlying adenocarcinoma (prevalence of secondary esophageal PD = 4.9%) and not with high grade dysplasia ("in situ" disease) or primary PD. A commonality among cases with Paget cells is the presence of focal or diffuse, poorly differentiated adenocarcinoma with discohesive cells. E-cadherin alterations also appear to play a role.

509 Quantitative Immunohistochemistry of Mast Cells in the Analysis of Colorectal Carcinogenesis

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Background: Mast cells have been implicated in tumor progression and prognosis in part because of their hypothesized role in angiogenesis. A significant increase in the number of mast cells has been observed in Min/+ mouse models of familial polyposis disease. Given the emerging link between cancer and inflammation and the recognized role of mast cells as central effectors in inflammatory reactions, the aim of this work was to study the potential role of mast cells in colorectal carcinogenesis by semi-quantifying the number of mast cells in premalignant conditions including tubular adenomas with/without high grade dysplasia and hyperplastic polyps in comparison with normal mucosa.
Design: Hyperplastic polyps (n=27), tubular adenomas (n=30), and tubular adenomas with high grade dysplasia (n=20) were selected from archived pathological specimens at our institution. Mast cells were detected immunohistochemically with mast cell tryptase antibody and the avidin-biotin-peroxidase approach. Mast cells were semi-quantified for at least ten microscopic fields at a magnification of 40X.
Results: Tryptase strongly labeled mast cells in the lamina propria of colonic mucosa and polypoid lesions. Positive staining was confined to the cytoplasm. Semi-quantitative analysis of mast cells was performed. In normal mucosa, the mean number of mast cells was 14.8 ± 3.7 (R: 11.7-22.3). In the premalignant conditions, the mean number of mast cells was 21.2 ± 7.1 (R: 10.0-39.8) in hyperplastic polyps, 21.2 ± 4.4 in tubular adenomas (R: 12.3-32), and 18.4 ± 4.2 in high grade tubular adenomas.
Conclusions: Our results demonstrate marked lamina propria infiltration by mast cells in hyperplastic and adenomatous polyps, suggesting mast cell-mediated inflammation may be involved in early-stage colorectal carcinogenesis. Further study of mast cell-mediated inflammation in colorectal carcinogenesis is warranted.

510 Overexpression of SmgGDS in Colon Carcinoma

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Background: SmgGDS is a guanine nucleotide exchange factor with the unique ability to activate multiple small GTPases, implicating it in cancer development and progression. We previously demonstrated that SmgGDS is overexpressed in prostate, breast and lung carcinomas, indicating a potential role as a diagnostic and prognostic marker in these tumors. We also showed that reduction of SmgGDS expression using siRNA in different cancer cells inhibits cell proliferation and reduces cell migration, further suggesting that SmgGDS may be a potential target in the treatment of these cancers. The expression and function of SmgGDS in colon cancer has not been reported. In this study, we evaluated the expression of SmgGDS in a large series of colon cancer cases including invasive carcinoma (InvCa), tubular adenoma/carcinoma in situ (TA/CIS) and metastatic carcinoma (Met) using immunohistochemistry (IHC).
Design: A total of 163 primary InvCa cases, as well as 53 TA/CIS and 38 Met identified from the 163 cases, were selected and stained with monoclonal anti-SmgGDS antibody. Tumors having stronger intensity and/or more area of staining were considered as overexpression when compared with the adjacent benign epithelium.
Results: 1. SmgGDS is overexpressed in colon cancers (see table 1). 2. Overexpression of SmgGDS is identified at different stages of tumor development in same patients. The expression is strongly correlated between InvCa and TA/CIS (n = 53, K = 0.89, p = 6.1E-09) and Met (n = 38, K = 0.58, p = 0.0003). 3. Expression of SmgGDS in colon cancer is independent of tumor stage and grade.

Overexpression of SmgGDS in Colon cancer

InvCa	TA/CIS	Met
123/163 (75.5%)	42/53 (79.2%)	31/38 (81.6%)

Conclusions: 1. SmgGDS is overexpressed in different stages of colon cancer, indicating a potential role as a diagnostic and prognostic marker of colon cancer. 2. The increased SmgGDS expression in TA/CIS suggests its role in early colon carcinogenesis. 3. These findings suggest that SmgGDS may hold value as a marker for tumor diagnosis or a target for the prevention and treatment of colon cancer.

511 p16INK4a Is Surrogate Marker of High-Risk HPV in High-Grade Squamous Lesions and Invasive Carcinomas of the Anal Canal, but Not in Anal Condyloma

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Background: p16INK4a is used as a surrogate marker for high-risk HPV (HR-HPV) in the uterine cervix, and has proven useful in the diagnosis of cervical squamous

intraepithelial lesions (SIL). However, the potential utility of p16INK4a in the diagnosis of SIL of the anal canal is less well-defined.
Design: We examined 78 anal lesions [14 non-dysplastic (acrochordons, hemorrhoids), 28 low-grade SIL (LSIL/AIN I/condyloma), 21 high-grade SIL (HSIL/AIN II-III/CIS) and 15 invasive squamous carcinomas] from 51 patients [32 male, 19 female; average age 42.7 years] to determine the pattern of p16INK4a (CINtec, mtm Laboratories) and Ki-67 (1:200, Dako) reactivity. p16INK4a was scored as negative (including blush or strong in <5% cells), focal strong (strong in 5-80% cells) or diffuse strong (strong in >80% cells). Ki-67 reactivity was scored as positive when staining in the upper 2/3 of the mucosa. HPV typing by PCR with pyrosequencing was performed on a subset of cases.
Results: p16INK4a reactivity and HR-HPV was observed in the majority of invasive carcinomas and HSIL. Two of the p16INK4a negative cases from this group were positive for HPV 16. In contrast, less than half of LSIL demonstrated p16INK4a reactivity, though the majority of these p16INK4a-negative LSIL (13/16) showed increased proliferation by Ki-67. In addition, HPV 16 was detected in all four of the p16INK4a-negative LSIL that were genotyped. 14/14 non-dysplastic lesions were negative for p16INK4a and Ki-67, and no HPV DNA was detected.

	p16 neg	p16 focal	p16 diffuse	Ki67	HR-HPV
Non-dysplastic	14/14	0/14	0/14	0/14	0/11
LSIL	16/28	10/28	2/28	22/28	4/4
HSIL	1/21	6/21	14/21	21/21	7/7
Invasive CA	3/15	1/15	11/15	13/15	12/12

Conclusions: Our results indicate that HR-HPV (most commonly HPV16) is present in the majority of high-grade lesions (invasive carcinomas and dysplasias) of the anal canal and in a subset of anal condylomas. In addition, immunohistochemical staining for p16INK4a showed focal or diffuse strong reactivity in the majority of the high-grade lesions but not in the low-grade lesions. While p16INK4a reactivity can be helpful in the diagnosis of high-grade lesions of the anal canal, it appears to have limited utility in the diagnosis of low-grade lesions, including lesions that harbor HR-HPV.

512 Investigation of the Molecular Mechanism of Esophageal Basaloid Squamous Cell Carcinoma

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Background: Basaloid squamous cell carcinomas (SCC's) of the esophagus are rare tumors that clinically behave in similar fashion to conventional esophageal SCC's. At other sites (e.g. oropharynx, anus) human papillomavirus (HPV) has been etiologically linked to tumors with a basaloid phenotype. HPV's role in esophageal SCC is controversial, and to our knowledge a direct examination of esophageal basaloid SCC's has not been previously performed. We examined the role of HPV and other molecular markers including p16, cyclin D1, and p53 in esophageal basaloid SCC.
Design: Nine cases previously diagnosed as esophageal basaloid SCC were retrieved from our surgical pathology files. Basaloid SCC was defined as a poorly differentiated SCC composed of nests of small cells, recapitulating the basal cell layer of non-neoplastic squamous epithelium. Twenty-two cases of conventional, keratinizing SCC's served as controls. In situ hybridization (ISH) for high-risk HPV was performed on the basaloid SCC's and three representative conventional SCC's. All cases were assessed for p16 (nuclear and/or cytoplasmic), cyclin D1 (nuclear), and p53 (nuclear) immunoreactivity. For each marker cases were scored based on the number of cells staining: 0 (0-5%), 1+ (5-25%), 2+ (25-50%), 3+ (50-75%), 4+ (75-100%). P53 staining intensity was additionally graded as weak, moderate, or intense.
Results: HPV ISH was non-reactive in all evaluated cases. The basaloid SCC's tended to be non-reactive with antibody to p16 (0 in 6, 1+ in 3), reactive with antibody to cyclin D1 (3+ in 1, 2+ in 2, 1+ in 4, 0 in 2), and reactive with antibody to p53 (4+ intense in 2, 3+ moderate in 2, 3+ weak in 1, 2+ weak in 1, 0 in 3). Conventional SCC's, in contrast, tended to be p16 immunoractive (20/22), generally with 1+, predominantly cytoplasmic staining at the periphery of invasive lobules. They tended to be cyclin D1 immunoreactive (3+ in 2, 2+ in 10, 1+ in 8, 0 in 2). P53 immuohistochemistry tended to mark a majority of tumor cells (3-4+) in intense fashion (4+ intense in 14, 3+ intense in 3, 4+ moderate in 1, 3+ moderate in 1, 4+ weak in 1, 3+ weak in 1, 2+ weak in 1).
Conclusions: Esophageal basaloid SCC's are high-risk HPV negative, distinguishing these lesions from other basaloid SCC's. Compared to conventional SCC controls, the basaloid SCC's show less p16 immunoreactivity and are less likely to manifest significant (3-4+ intense) p53 accumulation. As such, these lesions also appear molecularly distinct from conventional esophageal SCC's.

513 Practical Utility of Mucosal Eotaxin-3 mRNA Expression Levels in Diagnostically Challenging Cases of Reflux/Eosinophilic Esophagitis

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Background: The eosinophil chemotactic protein eotaxin-3 plays a central role in the pathogenesis of eosinophilic esophagitis (EE). Recently, eotaxin-3 mRNA was shown to be elevated in mucosal biopsies from patients with EE as opposed to those with GERD. Our aim was to explore the utility of eotaxin-3 mRNA expression in diagnostically challenging EE/GERD cases, and in EE patients who had undergone pre & post treatment biopsies.
Design: Twenty-four formalin-fixed biopsies belonging to 19 histologically equivocal cases, and 12 biopsies from five EE cases pre & post treatment were analyzed using RT-PCR. Equivocal cases were divided into two groups: patients with distal and mid/proximal biopsies containing 5-20 eosinophils/hpf (# 1), patients with distal biopsies containing >20 eosinophils/hpf with or without mid/proximal biopsies with 0-20 eosinophils/hpf (# 2).
Results: In the equivocal group 1, the mean eotaxin-3 level was 72.9 pg/ng. Using 99 pg/ng as the ROC cutoff (obtained from prior study with values >99 pg/ng in EE and <99 pg/ng in GERD), 4/12 fell in the EE and 8/12 in the GERD category. Group 2, had mean eotaxin-3 level of 66.62 pg/ng with 3/12 in the EE and 9/12 in the GERD category. Using this method, 4/4 patients clinically suspected of GERD had low eotaxin-3 levels. Of the remaining clinically and histologically equivocal cases 6/15 fell in the EE category (eotaxin-3 level 100.9-296.58 pg/ng). For the five EE cases for which pre and post EE therapy (3 steroids, 1 elemental diet, 1 PPI & antiallergy) biopsies were available, the mean eosinophil count dropped from 56.45/HPF to 18.3/HPF and eotaxin-3 level dropped from a mean of 1375.47 to 44.28 pg/ng (p=0.02). All patients had shown symptomatic improvement.
Conclusions: Our results show that eotaxin-3 mRNA may be a useful adjunct in histologically challenging cases and that eotaxin-3 level correlates with treatment outcome in patients with EE.

514 Epithelial-Mesenchymal Transition Markers in Pancreatic Ductal Adenocarcinoma

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Background: Transcription factors that mediate epithelial-mesenchymal transition (EMT) have been correlated with increasing histologic grade and suggested as useful tumor markers for some types of carcinoma. Pancreatic ductal adenocarcinoma (PDA), especially well-differentiated tumors, can be difficult to diagnose on core biopsy or FNA and more sensitive tumor markers are needed for early diagnosis of this disease. Therefore, expression of selected EMT markers were evaluated in a series of PDAs and compared to benign pancreas.
Design: A tissue microarray containing 76 cases of PDA of varying histologic grade, 13 cases of chronic pancreatitis, and 26 samples of normal pancreas was stained for Twist and Slug by immunohistochemistry. Staining indices (the product of a four-tiered scoring system for intensity and distribution) were recorded. Since indices of normal pancreas and chronic pancreatitis were not statistically different by Student's t-test, these cases were combined in a "Benign" group and compared to PDA of increasing grade by one-way ANOVA.
Results: Nuclear Twist and both nuclear and cytoplasmic Slug showed significant differences among the analyzed groups. Post-hoc analyses demonstrated that nuclear Twist was lower in all grades of PDA compared to benign pancreas (Grade 1, p= 0.0009; Grade 2, p= 0.0004; Grade 3, p= 0.0295). Nuclear Slug is significantly lower in grade 1 PDA compared to benign pancreas (t-test, p= 0.045); differences in higher grade PDA were not significant. Cytoplasmic Slug in low grade PDA is not significantly different than in benign pancreas, whereas it is decreased in higher grade PDA (Grade 2, p= 0.0065; Grade 3, p= 0.0066).
Conclusions: Patterns of IHC staining for the EMT markers Twist and Slug are significantly different in PDA compared to normal pancreas and chronic pancreatitis, and may be useful as diagnostic and prognostic markers.

IHC of EMT Markers in Pancreatic Ductal Adenocarcinoma

Twist (N)	**n**	**Mean**	**S.D.**	**Fisher F-value**	**Significance (p)**
Benign	43	4.42	1.83		
Grade 1	15	2.47*	1.92		
Grade 2	34	2.74*	2.12		
Grade 3	15	3.13*	2.17	6.290	<0.001
Slug (N)					
Benign	43	3.35	2.18		
Grade 1	16	2.06*	2.05		
Grade 2	35	2.67	2.04		
Grade 3	17	3.94	2.08	2.833	0.042
Slug (C)					
Benign	43	2.98	1.01		
Grade 1	16	2.44	0.89		
Grade 2	36	2.25*	1.30		
Grade 3	16	2.13*	1.09	3.841	0.012

* Statistically different compared to Benign (Student's t-test)

515 Role of MLH1/MSH2 Expression in Tumor Phenotype and Invasion in Stages 0 and I Sporadic Colorectal Adenocarcinomas

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Background: The role of DNA mismatch proteins on tumor cell kinetics is controversial, and no data are available for sporadic colorectal carcinoma (CRC) limited to the intestinal wall (distinction stage 0 vs. stage I).
Design: We selected B-RAF-wild sporadic CRC (40 stage 0 and 56 stage I) and analyzed necrosis, inflammatory response, growth pattern; mitotic figure counting, Ki-67 index, G2+M phase fraction, in situ end labeling (ISEL) of DNA fragments, and mlh-1/msh-2 immunoexpression. Variables were studied regarding mlh-1/msh-2 expression (positivity threshold 15%) and considered statistically significant if P<0.05. 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 the human GeneChip X3P Array noncompetitively. Cross-validated gene expression analyses (CGEA) were performed (expression factor>2, significance<0.01), and variables studied regarding the histological diagnosis (staging).
Results: Stage 0 adenocarcinomas less frequently revealed ulceration, necrosis and neutrophilic inflammation. MLH1/MSH2 expression was significantly lower in stage I adenocarcinomas (Table). MLH1/MSH2 immunoexpression>15% correlated with polypoid growth (P=0.03), absence of necrosis (P=0.04), and no inflammatory response (P=0.03). MLH1/MSH2 expression level showed direct correlation with mitotic figure counting, Ki-67 index, and G2+M phase fraction and inverse with ISEL index in stage 0 CRC only. CGEA revealed MLH1/MSH2 expression positively correlated with RAS homolog gene (member V), and PI3P phosphatase, and negative correlated with Trefoil factor 3, Caspase recruitment domain (member 9), and Meprin A.

Conclusions: MLH1/MSH2 expression in sporadic CRC results in down-regulated apoptosis (Caspase 9), inflammation (Trefoil factor 3), and stromal degradation (Meprin A), and up-regulated proliferation (RAS homolog and PI3P phosphatase activation of MAPK pathway). This gene profile explains both tumor phenotype, cell kinetic (proliferation, apoptosis) and helps in the CRC staging (stage 0 vs. stage I).

MLH1/MSH2 expression in CRC

	Stage 0	Stage I	Significance
MLH1 (%)	33.1	18.9	<0.001
MSH2 (%)	16.5	7.3	0.025

516 Hyperplastic Polyps in IBD: A Clinical, Pathologic and Molecular Study

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Background: Not uncommonly, patients with ulcerative colitis (UC) or Crohns colitis (CC) develop non-dysplastic serrated polyps histologically identical to microvesicular hyperplastic polyps (HP). Several previous studies have reported a substantial incidence of serrated dysplasia in IBD. The purpose of this study was to evaluate the clinical and pathologic features, natural history, and selected molecular abnormalities, of HPs in patients with UC or CC.

Design: The study consisted of 63 IBD patients (CD: 24, UC: 39, M/F = 17/36, mean age 54 years, mean disease duration: 159 months) who had 122 HPs (CD: 43, UC: 79). A wide variety of clinical and pathologic features (size, location either within or outside areas of colitis, development of further HPs or dysplastic polyps among others) were evaluated and compared between CD and UC patients, and between polyps located within vs. outside areas of colitis. A subgroup of 16 patients with paired colitic and HP mucosa were analyzed for B-RAF and K-RAS mutations.

Results: Overall, 61% of patients had 1 polyp, 23% 2 polyps, and 16% ≥ 3. 76% were located in the left colon; 24% in the right colon; mean size 2.8 mm (range 1-10). 76% occurred in areas of underlying chronic, or chronic active, colitis. Follow up was available in 40% of patients (mean: 20.2 months). 96% developed subsequent HPs (75% in the same location, 25% in other locations). 16% of patients developed an adenoma-like DALM, and none developed flat low-grade dysplasia. 6 patients (of 63) (9.5%) had colon cancer, either prior to (N = 3) or at the time (N = 3) of the HP diagnosis. 3 of 16 (19%) cases showed B-RAF mutations in one of their HPs (UC: 2, CD: 1) that were not present in the background colitic mucosa, and none showed KRAS mutations. There were no significant differences with regard to any of the clinical or pathologic features between CD or UC patients with HPs, or between HPs located within vs. outside areas of colitis.

Conclusions: Similar to sporadic HPs in the general population, HPs in patients with IBD occur predominantly in the left colon, may be single or multiple, and have a benign natural history. They also show a low association with B-RAF and K-RAS mutations. The similar characteristic of HPs in CD and UC patients, and of HPs both within, and outside, areas of colitis, suggests that these polyps most likely develop sporadically, and unrelated to the patients underlying colitis.

517 Neural Tumors of the Gastrointestinal Tract: A Case Series of 59 Tumors

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Background: The initiation of upper endoscopy and widespread colonoscopic screening has resulted in the identification of a variety of different types of neural tumors of the gastrointestinal tract (GI), but the clinicopathologic presentation of this group of lesions has not been well defined.

Design: Demographic and morphologic features of 59 biopsies from 54 patients diagnosed with neural GI tumors from 1993-2007 at 3 institutions were evaluated. Immunohistochemical stains were performed when necessary. Specific features included (1) age at diagnosis, (2) location within the gastrointestinal tract, (3) morphology (spindled, gangliocytic, or mixed), (4) location within the mucosa and/or submucosa, (5) size, and (6) presence or absence of an inflammatory infiltrate.

Results: The mean age at diagnosis was 53.4 years. The colon was most commonly affected (76%), followed by stomach (7%), esophagus (5%), small bowel (3.5%), and rectum (3.5%). Location was unavailable for 5% of cases. Most involved mucosa and submucosa, and although most were not removed with clear margins, 78% (46/59) were less than or equal to 5 mm on endoscopy. Ganglioneuromas were the most common neural lesion, accounting for 59% (35/59) of lesions. Granular cell tumors accounted for 12% (7/59), mucosal neuromas for 10% (6/59), Schwannomas for 10% (6/59), Perineurinomas for 5% (3/59), and Neurofibromas and Gangliocytic paragangliomas each accounted for 2% (1/59). Most Ganglioneuromas were sporadic; 20% (7/35) occurred in 3 patients with an intestinal ganglioneuromatosis-associated syndrome; and 49% (17/35) were associated with mucosal or submucosal lymphoid nodules.

Conclusions: Submucosal and mucosal neural lesions in the GI tract are often identified at first screening colonoscopy in patients over 50 years of age. Ganglioneuromas are the most common, usually sporadic, and often associated with lymphoid nodules in approximately one-half of cases. This association is of unclear significance and deserves further study. Pathologists should consider ganglioneuroma when making the diagnosis of a benign lymphoid aggregate in the lower GI tract.

518 The Role of Tumour Perforation in Dukes B Colonic Carcinoma

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Background: Peritoneal involvement has consistently emerged as an independent prognostic factor in colonic carcinoma. A common feature of this malignancy is the presence of peritumoural inflammation, which potentially compromises the peritoneal barrier resulting in tumuor perforation and dissemination into the peritoneal cavity. The aim of this study was to characterise tumour perforation in Dukes B colonic carcinoma with regard to direct or indirect inflammatory peritoneal involvement.

Design: All pT_4N_0 colon carcinomas over an eleven-year period were selected from the colorectal cancer database at this institution between January 1996 and December 2006. Synchronous and metachronous tumours, those arising in UC or FAP and patients receiving neoadjuvant chemoradiotherapy were excluded. A total of 78 patients were identified. H&E sections were examined without knowledge of patient outcome. Pathological parameters evaluated included tumour perforation (microscopic and macroscopic), peritumoural inflammation and microabscess formation, tumour budding, lymphovascular invasion (LVI), perineural invasion and tumour margin. Survival was correlated with the above features.

Results: The study population comprised 47 (60%) males and 31 (40%) females aged between 23 & 91 years. Perforated tumours accounted for 1/3 of all T4 tumours. They were more common in the left colon (p=0.007), were less likely to have an infiltrative margin (p=0.008) and showed LVI less frequently (p=0.02). The median follow-up was 1.66 years. 25 deaths have been recorded, 19 of which were cancer-related. 5 perforated tumours showed direct peritoneal spread to adjacent organs (T4a), with 3 such cases resulting in patient death. There was little variation in mortality between perforated (19%) and non-perforated (21%) tumours.

Conclusions: Although showing more favourable pathological characteristics (less LVI, and an expansile margin), perforated colonic tumours show a similarly poor prognosis to tumours with direct peritoneal extension without perforation.

519 Aberrant CDX2 and CK7/CK20 Expression in Colorectal Adenocarcinomas with High-Frequency Microsatellite Instability (MSI-H)

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Background: The MSI pathway is involved in hereditary nonpolyposis colorectal carcinoma (HNPCC) and ~15% sporadic colorectal adenocarcinomas (CRCs). Most microsatellite stable (MSS) CRCs are characterized by a CK7-/CK20+/CDX2+ immunophenotype (IP). Aberrant CK7+/CK20- expression has been reported in MSI-H CRC, sometimes leading to confusion between primary CRC and metastasis to the colon, especially in poorly differentiated tumors. In this study, we investigate the expression of CK7, CK20, and CDX2 in MSI-H CRC and the utility of MSI markers in evaluating tumors of questionable origin.

Design: We studied 91 CRCs with known MSI status including 70 MSI-H (36M:34F; 30-91 yrs; mean 59 yrs) and 21 MSS (8M:13F; 34-76 yrs; mean 49 yrs). MSI status was defined by NCI criteria based on PCR amplification of 5-10 microsatellite markers. Tumors were immunostained for CK7, CK20, CDX2, and the DNA mismatch repair enzymes MLH1, MSH2, MSH6, and PMS2. For CDX2 and CK20, any labeling of tumor cells was classified as positive. For CK7, positivity was defined as aberrant labeling in >5% tumor cells. Expression of DNA mismatch repair enzymes was either positive (normal result) or negative.

Results: MSI-H CRCs were more likely to have aberrant loss of CDX2 (p=0.03) and CK20 (p=0.003) and to have +CK7 expression (p=0.03) than MSS CRCs. 90% (19/21) MSS tumors showed the usual CK7-/CK20+/CDX2+ IP, while 10% (2/21) were CK7+/CK20+/CDX2+. In contrast, only 50% (35/70) MSI-H tumors had the usual CK7-/CK20+/CDX2+ IP (p<0.001). Other IPs in MSI-H tumors were as follows: 18 CK7+/CK20+/CDX2+, 6 CK7-/CK20-/CDX2-, 3 CK7+/CK20-/CDX2-, 3 CK7-/CK20+/CDX2-, 2 CK7-/CK20-/CDX2+, 2 CK7+/CK20-/CDX2+, and 1 CK7+/CK20+/CDX2- (many of which could cause confusion with other primaries). There was no significant correlation between the tumor's IP and loss of MLH1/PMS2 vs loss of MSH2/MSH6.

	CK7		CK20		CDX2	
MSI Status	+	-	+	-	+	-
MSI-H (n=70)	34%	66%	81%	19%	81%	19%
MLH1/PMS2 loss (n=48)	35%	65%	75%	25%	75%	25%
MSH2/MSH6 loss (n=19)	37%	63%	95%	5%	95%	5%
MSS (n=21)	10%	90%	100%	0%	100%	0%
p value	0.03		0.003		0.03	

Conclusions: Aberrant CK7/CK20/CDX2 IPs occur frequently (50%) in MSI-H CRC. These results indicate that MSI-H CRC should always be considered in the differential of primary and metastatic tumors. In the setting of an abnormal IP, MLH1/PMS2 or MSH2/MSH6 loss by immunostaining can help point to a MSI-H CRC primary.

520 Adequacy of Lymph Node Retrieval in Colonic Adenocarcinoma Depends on Surgical and Anatomic Factors

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Background: Pathologic staging of colorectal specimens submitted for malignancy mandates thorough examination and submission of all lymph nodes identified. A consensus statement by the College of American Pathologists (CAP), published in 1999, recommended that when fewer than 12 lymph nodes were retrieved that enhancement techniques should be employed. Various studies have shown that complete processing and evaluation of at least 12 lymph nodes limits the phenomenon of stage migration. Despite adherence to these CAP recommendations, lymph node retrieval may still be less than the number 12. This may be due to one or more of the following variables: patient

age, gender, race, site of tumor, surgical technique/length of specimen submitted, and training of the dissector (pathologist assistant (PA) versus pathology resident (MD)).
Design: Information on pathologic characteristics and patient demographics was obtained from institutional surgical reports and medical records of all patients with a resection for colonic malignancy between 1995 and 2006. From 457 cases identified, the following information was obtained: patient age, race, gender; dissector (PA vs. MD); specimen site, length, and number of nodes retrieved; and year of resection. Specimens were classified as "adequate" (≥12 nodes retrieved) or "inadequate" (<12 nodes retrieved). Multiple logistic regression was performed to identify factors which predicted adequate node retrieval.
Results: Of all cases analyzed, 278 (61%) had adequate node retrieval (≥12). Multiple logistic regression identified the following predictive factors: greater specimen length ($p<0.001$), more proximal tumor site ($p<0.001$), younger patient age ($p<0.01$), and more recent year of dissection ($p<0.005$). There was no statistically significant association with patient race or gender or dissector experience (PA vs. MD).
Conclusions: Younger patient age, proximal tumor site, longer specimen length, and more recent procedural date were found to be independent, positive predictive factors for "adequate" (≥12) lymph node retrieval in colon cancer resections. Resections less than 15 cm in length as well as resections from the sigmoid colon and rectum were associated with a less than 50% rate of "adequate" lymph node retreival (<12). Based on these findings, the definition of "adequate" lymph node retrieval might be refined to consider surgical and anatomic factors.

521 Are Tumefactive Lesions Classified as Sclerosing Mesenteritis [Mesenteric Panniculitis, Mesenteric Lipodystrophy] a Subset of IgG4-Related Sclerosing Disorders?

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Background: Sclerosing mesenteritis (mesenteric panniculitis, mesenteric lipodystrophy) has been regarded as a disorder distinct from retractile mesenteritis (retroperitoneal fibrosis, Ormond disease), but the relationship between these disorders is debatable. Recently, a family of diseases unified by IgG4 expression has been delineated encompassing primarily lymphoplasmacytic sclerosing pancreatitis (LPSP), sclerosing cholangitis, idiopathic retroperitoneal fibrosis (Ormond disease), and chronic sclerosing sialadenitis (Kuttner tumor). The prototype disease in this category (LPSP) is characterized by elevated serum IgG4 and IgG4 expression in lesional plasma cells, obliterative lymphocytic phlebitis, and response to steroid treatment.
Design: We reviewed the clinicopathologic features of mass lesions identified from our files as "sclerosing mesenteritis". Inclusion in the study required available paraffin blocks in order to assess IgG4 expression by immunohistochemistry. IgG4-positive plasma cells were counted in the areas of highest density in at least 3 high power fields (HPFs). The highest number per HPF was recorded and a score was assigned based on a previously published method: <5/HPF- none/minimal; 5-10/HPF- mild; 11-30/HFP- moderate; >30/HPF- marked. EBER labeling was performed in 5 cases.
Results: There were 7 mesenteric lesions, ranging in size from 5 to 14 cm (median 8 cm), from 4 men and 3 women. All were characterized as a loosely marginated mass-forming fibroinflammatory process with variable fat necrosis. On IgG4 immunohistochemistry, reactive plasma cells ranged in number from 0 to >100 in the densest zones (2 cases – absent or minimal, 3 cases - moderate, 2 cases – marked). Of interest, 5/7 displayed lymphocytic/obliterative phlebitis indistinguishable from that observed in lymphoplasmacytic sclerosing pancreatitis. None of the lesions involved the pancreas. All 5 tested cases were EBER negative. On follow-up, available for 5 patients, 2 died of unrelated causes (both at 4 mos). The others were alive with no disease or stable disease at 3 mos, 5 years and 15 years.
Conclusions: Although the study is limited by small numbers, our findings suggest that at least a subset of tumefactive lesions regarded as sclerosing mesenteritis (mesenteric panniculitis, mesenteric lipodystrophy) are unified with other reactive IgG4-related sclerosing disorders by the presence of both lymphocytic phlebitis and prominent IgG4-expressing plasma cells, possibly accounting for a response to steroids in a subset of patients.

522 Is IgG4 Specific for Lymphoplasmacytic Sclerosing Pancreatitis and Similar Fibroinflammatory Lesions?

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Background: Prominence of IgG4-positive plasma cells has been described in association with a number of lesions sharing the features of fibrosis, lymphocyte and plasma cell-predominant chronic inflammation and in some cases, obliterative phlebitis, the prototype of which is lymphoplasmacytic sclerosing pancreatitis (LPSP). To address the specificity of IgG4 staining in our material, we selected entities with an established association with IgG4-immunolabeled plasma cells and those for which the association has been less extensively studied.
Design: Hematoxylin and eosin (H&E)-stained slides and paraffin blocks were obtained from the Pathology archives of a large academic hospital for the following diagnoses: LPSP, chronic pancreatitis NOS, inflammatory pseudotumor/inflammatory myofibroblastic tumor (IPT/IMT), sclerosing mesenteritis (SM), ulcerative colitis, and Crohn's disease. H&E slides were reviewed and immunohistochemical staining for IgG4 was performed on a representative block from each case. IgG4-positive plasma cells were counted in the areas of highest density for at least 3 high power fields (HPFs). The highest number per HPF was recorded, and a score was assigned based on a previously published method: less than 5/HPF- none/minimal; 5-10/HPF- mild; 11-30/HFP- moderate; greater than 30/HPF- marked.
Results: Prominent IgG4 labeling was encountered in most examples of LPSP but also in a subset of the other entities (see table). Fewer IgG4-positive plasma cells in cases of LPSP correlated with increased sclerosis and less abundant chronic inflammation. However, IgG4 staining was not consistently proportional to the degree of inflammation in other lesions.

IgG4 Labeling in Various Conditions

Entity	Total Cases	None/Minimal	Mild	Moderate	Marked
LPSP	25	0 (0%)	1 (4.0%)	3 (12.0%)	21 (84.0%)
Pancreatitis, NOS	9	5 (55.6%)	3 (33.3%)	0 (0%)	1 (11.1%)
IPT/IMT	22	14 (63.6%)	2 (9.1%)	3 (13.6%)	3 (13.6%)
SM	8	3 (37.5%)	0 (0%)	3 (37.5%)	2 (12.5%)
Ulcerative Colitis	9	1 (11.1%)	1 (11.1%)	3 (33.3%)	4 (44.4%)
Crohn's disease	7	0 (0%)	1 (14.3%)	6 (85.7%)	0 (0%)

Conclusions: IgG4 labeling is a sensitive but not specific marker in LPSP. It may be less prominent when sclerosis predominates over inflammation. In our material, IgG4 staining of IPT/IMT and sclerosing mesenteritis was variable. A range of IgG4-labeling was observed in idiopathic inflammatory bowel disease. The degree of IgG4 labeling may not parallel the overall density of inflammation in some lesions.

523 Pyloric Gland Adenoma (PGA): An Entity Distinct from Gastric Type Adenoma (GA)

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Background: Abraham et al (Am J Surg Pathol 2002; 26: 1276) have divided gastric adenomas into intestinal and gastric types. The gastric type adenomas display an apical cap of neutral mucin without intestinal metaplasia (IM) whereas the intestinal type has IM. Gastric type adenomas are significantly less likely to show high-grade dysplasia, adenocarcinoma within the polyp, and gastritis with IM in the surrounding stomach. Pyloric gland adenoma displays gastric type epithelium but lacks a neutral mucin cap. Initially described in the 70's, it has not been well recognized as a distinct entity, particularly in the US, despite the series by Vieth et al (Virchows Arch, 2003, 442:317). We evaluated clinical and histopathological features of pyloric gland adenoma in comparison with conventional gastric type adenoma.
Design: We reviewed 35 PGA from 31 patients and 28 GA from 24 patients and labeled a subset (9 PGA and 7 GA) with an immunohistochemical panel of mucin core peptides (MUCs) and CDX2.
Results: PGA involved the duodenum (17) or stomach (18) of 21 F and 10 M ranging in age from 51-89 yrs (median, 72 y). On H&E stains, PGA displayed cells lacking an apical mucin cap. Cells were arranged as closely packed deep pyloric gland-type tubules with a monolayer of cuboidal to low columnar epithelium with pale to eosinophilic cytoplasm with a ground glass appearance. In gastric lesions, the background mucosa had IM in a subset of cases. Associated invasive carcinomas were reviewed in 2 cases. On immunohstochemical staining lesions expressed MUC6 (9/9, a pyloric gland mucin marker), with co-expression with MUC5AC (9/9, a foveolar mucin marker). In most of the cases, MUC5AC stained the superficial portion of the lesions. Intermixed staining with MUC6 was observed in 6 PGA. Except a single case displaying focal labeling, all lesions lacked MUC2 (8/9, an intestinal mucin marker) and CDX2 (8/9). Both associated carcinomas coexpressed MUC5AC and MUC6. In contrast, GA involved the stomach (28) of 12 F and 12 M aged 18 to 80 (median, 44 y). The lesions were composed of cells with elongate stratified nuclei and the typical apical mucin cap unaccompanied by background IM. On immunohistochemistry, the cells labeled with MUC5AC (7/7) but lacked MUC2 (0/7), and CDX2 (0/7). One case had focal MUC6 (1/7).
Conclusions: As noted by others, PGA is a distinct entity. Its appearances are deceptively bland but, in contrast to GA, it is likely to be accompanied by background IM and can be associated with adenocarcinomas displaying similar differentiation.

524 Application of a Global Proteomic Approach to Archival Precursor Lesions: Upregulation of DMBT-1 and TG2 in Pancreatic Cancer Precursors

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Background: Pancreatic cancer is a fatal disease; hence, early detection is a critical determinant of improved survival. A variety of non-invasive precursor lesions of pancreatic adenocarcinoma have been identified, which provide a unique opportunity for intervention prior to onset of invasive cancer. Biomarker discovery in precursor lesions has been hampered by the ready availability of fresh specimens, and limited yields of nucleic acids or proteins suitable for large scale screening.
Design: Therefore, we decided to use the Liquid Tissue®, a novel technique for protein extraction from archival formalin-fixed material, in order to perform global proteomic analysis of an intraductal papillary mucinous neoplasm (IPMN), a non-invasive pancreatic cancer precursor. Approximately, 30,000 epithelial cells were collected from an archival IPMN by manual microdissection, and a soluble Liquid Tissue® lysate representative of the total protein complement was prepared. 500ng of the protein extract was analyzed by High resolution liquid chromatography and tandem mass spectrometry (LC-MS/MS). In addition, tissue microarrays (TMAs) comprised of 35 archival IPMNs was used for validation of candidate markers by immunohistochemistry.
Results: The LC-MS/MS of the IPMN Liquid Tissue® lysate resulted in the identification of 3545 peptides corresponding to 399 unique proteins (range of 1 to 16 peptides for each protein). Manual curation established that a subset of proteins expressed in the non-invasive IPMN had been reported as upregulated in invasive pancreatic cancer by previous mRNA and protein expression profiling studies. From this list, we decided to perform immunohistochemical labeling for two of the proteins in this "enriched" pancreatic cancer-associated subset - Deleted in Malignant Brain Tumors (DMBT1) and Tissue Transglutaminase (TG2) - confirmed their overexpression in archival IPMN tissues.

Conclusions: Global proteomics analysis using Liquid Tissue® reagents and LC-MS/MS is a feasible approach for unbiased biomarker discovery ("forward proteomics") in limited archival material, particularly applicable to precursor lesions of cancer.

525 Overexpression of GADD 45α in Colorectal Adenocarcinoma

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Background: The growth arrest and DNA damage-inducible 45α gene (GADD45α) is one of the downstream mediators of p53 that is induced in response to DNA damage. Previous studies have identified GADD45α as a possible mediator in the carcinogenesis and progression of colorectal cancer. The present study was designed to assess the clinicopathological significance of GADD45α expression in colorectal adenocarcinoma.

Design: Formalin-fixed, paraffin-embedded sections from 97 colorectal adenocarcinomas (CRCs) were immunostained by an automated method (Ventana Medical Systems; Tuscon, AZ) using rabbit polyclonal GADD45α antibody (sc-792; Santa Cruz Biotechnology, Santa Cruz, CA). Cytoplasmic immunoreactivity was semiquantitatively evaluated based on both intensity and distribution and results were correlated with histologic and prognostic variables.

Results: Overexpression of GADD45α was observed in 65/97 (67%) CRCs and correlated with LN status (80% node negative vs. 56% node positive, p=0.014) and showed a trend toward overexpression in tumors without mucin production (74% without mucin vs 56% with mucin, p=0.07). Intense, diffuse overexpression was observed in a subset of 25/97 (26%) tumors and correlated with LN status (37% node negative vs. 14% node positive, p=0.013) and survival (32% expired vs. 5% alive, p=0.010). Within the LN negative subgroup, intense, diffuse overexpression GADD45α correlated with tumor stage (48% advanced stage vs. 18% early stage, p=0.048) and survival (53% expired vs. 6% alive, p=0.002).

Conclusions: GADD45α overexpression is associated with tumor aggressiveness in CRC and is associated with tumors that feature an aggressive clinical course despite node negative status. Given the potential association of GADD45αexpression with resistance to radiation and platinin-based chemotherapy, further study of this biomarker in CRC patients appears warranted.

526 Neoplasia of the Pouch and Anal Transitional Zone in Patients with Ulcerative Colitis Treated by Ileal Pouch-Anal Anastomosis: A Morphologic Study of a Rare Group of Neoplasms

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Background: Patients with ulcerative colitis (UC) have a high lifetime risk for developing colorectal dysplasia and carcinoma. In UC patients who have undergone proctocolectomy with ileal pouch-anal anastomosis (IPAA), this risk, although minimized, does not completely disappear, even with handsewn anastomosis with mucosectomy. The morphologic features of such neoplasms arising in the anal transitional zone or, even more rarely, in the pouch itself, have not been well described.

Design: 2515 patients who underwent restorative proctocolectomy with or without anal transitional zone (ATZ)-sparing IPAA for UC or indeterminate colitis between 1983 and 2007 were included. Serial ATZ and pouch biopsies were taken for at least 2 years after ileostomy take-down. Mean follow-up was 112 (range 24-228) months.

Results: We identified 9 cases of adenocarcinoma in 2515 patients with ileal pouches for UC or indeterminate colitis (prevalence=0.36%): cancer in the ATZ (6) and/or pouch (3), 7M:2F, mean age 58.2 years (range 36-82). Of the 9 original proctocolectomy specimens, only 1 already had carcinoma in the rectum and 1 had HGD. Mean detection time for cancer after IPAA was 103 (range 0-240) months. Dysplasia was found prior to cancer detection in post-IPAA biopsies in 5 patients (83%): low grade (LGD) (1), high grade (HGD) (1), LGD and HGD (3). Mean detection time for dysplasia after IPAA was 97.8 (range 0-228) months. Three patients with HGD in the ATZ or pouch were later found to have adenocarcinoma. Adjacent dysplasia was found at resection in 6 patients (67%): HGD (3), LGD and HGD (3). In 4 cases, prior to and/or at the time of cancer resection, the dysplasia showed a villiform architecture with hypermucinous features; 4 adenocarcinomas demonstrated mucinous features. Two patients showed dysplasia with hypermucinous features in post-IPAA biopsies up to two years prior to development of carcinoma in the ATZ (1) and pouch (1).

Conclusions: While the incidence of ATZ and pouch adenocarcinomas may be low, the finding of dysplasia adjacent to the carcinomas or in previous biopsies highlights the need for long term surveillance following IPAA. Although our sample is limited, the higher incidence of pre-cancer dysplasia with hypermucinous features, distinct from traditional dysplasia but similar to the carcinomas, reinforces a need for recognizing this type of epithelium. Future studies with longer follow-up are necessary.

527 Predictive Value of Mitosin and CDC2 Expression in the Development of Adenocarcinoma in Barrett's Esophagus

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Background: Aneuploidy, by flow cytometry, has been shown to have strong predictive value for the development of esophageal adenocarcinoma (EAC) in Barrett's esophagus (BE). We have previously shown that the expression of mitosin (CENPF) and CDC2 correlates with the presence of aneuploidy in patients with BE and related neoplastic lesions. The aim of this retrospective case-control study was to evaluate the utility of these two potential biomarkers in predicting risk of cancer in BE.

Design: Thirty-five patients with BE who had undergone surveillance endoscopies were identified from two hospitals between 1990 and 2003 (mean age; 66 years, all male). During the course of surveillance, 12 progressed to EAC whereas 23 did not. The mean duration of follow-up was 30 months in the EAC group and 81 months in the non-EAC groups. The presence and degree of immunohistochemical staining for mitosin and CDC2 were evaluated in the index esophageal mucosal biopsy for each patient, in a blinded fashion, without knowledge of outcome. The results (expressed as % positive cells/total cells) were compared between the two patient groups.

Results: In the index biopsies of BE, mitosin expression was significantly (p=0.02) increased in patients who developed cancer (13.21% ± 3.09) compared to those who did not (9.90% ± 4.67). Although CDC-2 expression was increased in the EAC group (2.14% +/-5.15 vs. 0.83% +/-2.27), these values did not reach statistical significance (p=0.41). Neither mitosin, nor CDC2, expression correlated with grade of dysplasia in patients who developed this neoplastic change during the course of surveillance.

Conclusions: Immunohistochemical expression of mitosin occurs early in the progression of neoplasia, prior to the onset of dysplasia, and may be useful to help predict high risk BE patients prone to develop adenocarcinoma. Future prospective studies on a larger group of patients should be performed to confirm these findings.

528 BRAF (V600E) Mutation and Microsatellite Instability in Crohn's Disease Associated Adenocarcinoma

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Background: Surveillance of patients with inflammatory bowel disease (IBD) currently revolves around detection of "adenomatous" dysplasia as a marker of increased risk of adenocarcinoma. Recent studies of sporadic colorectal cancer (CRC) have shown BRAF (V600E) mutation to be a marker of a second pathway of carcinogenesis that originates in a hyperplastic polyp and culminates in MSI-high CRC. The prevalence of BRAF mutation in CRC in IBD is thus indirect evidence of the frequency with which colon cancers arise from serrated "non-adenomatous" precursors in these patients. BRAF mutation occurs in about 9% of ulcerative colitis (UC) associated cancers but there is no data on its prevalence in Crohn's disease (CD) and was thus the aim of our study.

Design: 21 cases of CRC in CD were identified from the surgical pathology files of Massachusetts General Hospital. All patients had cancer in an area of chronic colitis and did not have a family history of colon cancer. DNA extraction was performed using a Qiagen tissue extraction protocol on paraffin embedded tissue. The BRAF mutation in exon 15 (V600E) was assayed using a real time allele specific PCR. Control DNA included a negative control from normal lymph node and a positive control from a melanoma cell line. Immunohistochemistry for MLH1 and MSH2 was performed using commercially available antibodies.

Results: Successful DNA extraction was achieved in 17/21 cases as measured by spectrophotometry and a beta actin PCR. All 17 cases were negative for the BRAF mutation (V600E) by real-time allele specific amplification and showed a normal genotype for the BRAF gene. Immunohistochemical analysis showed complete loss of nuclear staining for MLH-1 in only one of these 17 (5.8%) cases. MSH-2 staining was intact in all cases.

Conclusions: The prevalence of BRAF (V600E) mutation in CD associated adenocarcinomas appears to be much lower than that seen in sporadic or UC related CRC. Loss of staining for MLH-1, consistent with MSI, in a subset of CD associated carcinoma suggests that the importance of serrated non-adenomatous lesions as precursors of CRC in these patients needs to be evaluated in future prospective studies.

529 Rare High Grade Sarcoma Arising in or near the Gastrointestinal Tract May Mimic Gastrointestinal Stromal Tumor (GIST) Histologically and Immunohistochemically

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Background: High grade sarcomas other than GIST that arise in or near the gastrointestinal tract may mimic GIST morphologically. We encountered a case of dedifferentiated liposarcoma of the gastrointestinal tract that closely resembled GIST and stained positive for CD117. A small study in the literature reported up to 30% of dedifferentiated liposarcoma showed focal immunoreactivity for CD117. However, no systematic study of the expression of GIST markers in gastrointestinal tract sarcomas has been conducted. We investigated the expression of CD117 and CD34 in a cohort of high grade abdominal and retroperitoneal non-GIST sarcoma cases.

Design: Twenty cases (2001-2007) met the selection criteria of subtyped non-GIST high grade sarcomas in or near the GI tract. Focally these sarcomas had histological features that mimicked spindle and /or epithelioid GIST. One representative block from each case was used for immunostaining for CD117 and CD34.

Results: Of the 20 patients, 13 were female and 7 were male with a median age of 62 years (ranging from 29 to 90 years). The diagnosis included leiomyosarcoma (n=5), high grade or dedifferentiated liposarcoma (n=10), malignant fibrous histiocytoma (n=4), and uterine stromal sarcoma (n=1). Eight cases had de novo diagnosis while 12 re-excision cases had a previous diagnosis of non-GIST sarcoma. Six sarcomas involved the gastrointestinal tract, eight arose in the retroperitonium, three arose in the abdomen, and three involved the liver. Nine (9/20, 45%) cases stained positive for CD34. CD117 staining was strong only in the index case (1/20, 5%) of recurrent myxoid liposarcoma with dedifferentiation involving the stomach and small bowel. CD117 was negative in the remaining 19 cases.

Conclusions: High grade sarcomas in the abdominal and retroperitoneal area may mimic GIST morphologically. These cases are frequently positive for CD34 but rarely positive for CD117. Dedifferentiated liposarcoma can pose a diagnostic challenge mimicking GIST in its staining pattern and may be a diagnostic pitfall when examining these cases.

530 Selective Internal Radiation Therapy-Induced Extrahepatic Injury: An Emerging Cause of Iatrogenic Organ Damage

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Background: Selective internal radiation therapy (SIRT) using 90-Yttrium labeled microspheres injected into the hepatic artery is an emerging regional approach to the treatment of primary and metastatic hepatic malignancies. It is advantageous because it allows for selective delivery of higher doses of radiation to tumors than would otherwise be tolerated by the surrounding hepatic parenchyma. Complications due to SIRT often result from inadvertent extrahepatic embolization of radioactive microspheres causing radiation-induced tissue injury. As very little has been written about this topic from a pathologic perspective we herein report our experience with extrahepatic SIRT injury, including 1 case originally misdiagnosed as adenocarcinoma.

Design: 4 cases of SIRT-induced extrahepatic tissue injury were identified in the consultative (2) and inpatient (2) files of our cancer institute. Clinical data, final pathology reports and accompanying H & E-stained slides were reviewed.

Results: 4 patients underwent SIR therapy for hepatic metastases from rectal adenocarcinoma (1), islet cell tumor (1) and carcinoid tumor (2). They each presented several months after treatment with abdominal pain. 1 patient was found to have cholecystitis on CT scan and underwent cholecystectomy, while upper GI endoscopy on the other 3 revealed antral ulcers that were biopsied. In each of the cases, microscopic examination showed SIRT microspheres predominantly located in small blood vessels but also scattered throughout the mucosa. The histologic appearance varied from acute injury and marked epithelial atypia that was originally misinterpreted as adenocarcinoma in 1 case to a chronic fibrous and granulomatous response.

Case No.	Age/Gender	Liver Metastasis Origin	Time post Rx (months)	Pathology
1	70/M	Rectal adenocarcinoma	2	Gastric ulcer
2	59/F	Carcinoid tumor	5	Gastric ulcer
3	68/F	Carcinoid tumor	11	Gastric ulcer
4	75/F	Islet cell tumor	1.5 and 2.5	Cholecystitis

Conclusions: Pathologic appearances of SIRT-induced tissue injury are variable and may mimic malignancy. Awareness of this emerging iatrogenic disease process is important for surgical pathologists, particularly to avoid overdiagnosis.

531 Event Rate over a 15-Year Period in a Regional Veteran Affairs Barrett's Cohort without Baseline Dysplasia

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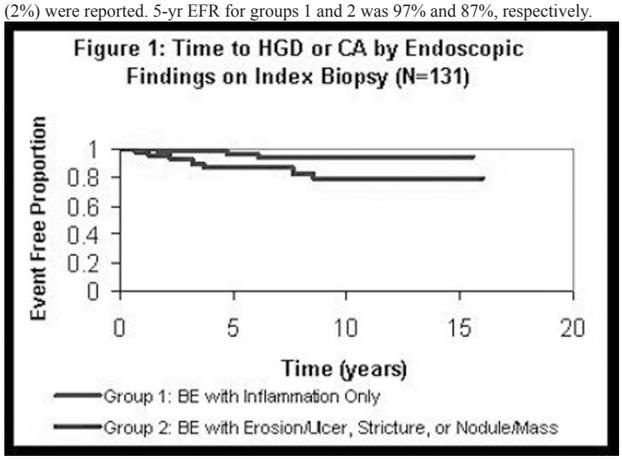
Background: Limited data is available that focuses on Barrett's esophagus (BE) without dysplasia. ACG '02 guidelines recommend 3-yr surveillance endoscopy for BE with 2 biopsies (bxs) negative for dysplasia (NEG). We evaluated a NEG-BE cohort for risk and time-to-event (TTE) rate with long term follow-up (f/u).

Design: All BE cases from 7/90-12/95 were taken from database and medical records were retrospectively reviewed through 6/06 to select cases NEG on 1st bx and with at least 1 f/u bx. Bxs were categorized as NEG, indefinite (INDF), low-grade dysplasia (LGD), high-grade dysplasia (HGD), or carcinoma (CA). 'Event' was defined as progression to HGD or CA. Endoscopy data was obtained on 1st bx. Statistical analysis included Fisher's exact and Kruskal-Wallis tests. Kaplan-Meier method was used to estimate event free rates (EFRs), with comparisons between groups performed with log-rank test.

Results: 131 patients (pts) underwent 699 bxs with 859 pt-yr f/u. 11 events occurred (7 CA; 4 HGD) with a rate of 1.3 events/100 pt-yrs. Almost all pts were males; mean age 65.4 yrs (R=35-83). Indications for bx were history of BE (34%), dysphagia (22%), abdominal pain (15%), bleeding/anemia (13%), heartburn (9%), ulcer/stricture (5%), and nausea/vomiting (2%). 58% had hiatus hernia. Mean BE length was 8.3 cm (R=1-21). BE with inflammation only (41%), stricture (29%), ulcer/erosion (28%), and nodule/mass (2%) were reported. 5-yr EFR for groups 1 and 2 was 97% and 87%, respectively.



10 of 15 pts who developed LGD, subsequently showed regression. 5-yr EFR was better when 2nd bx had INDF or LGD than NEG.

Table 1

Highest Subsequent Grade; N=131 (#/%)			
NEG		84/64.1	
INDF		21/16	
LGD		15/11.5	
HGD		4/3.1	
CA		7/5.3	
TTE Analysis by 2nd Bx (N=129)	EFR (%)		
	1 Yr	3 Yrs	5 Yrs
NEG	99	98	94
INDF	100	100	100
LGD	100	100	100

Of 11 events, 8, 0, and 1 events occurred when 2nd bx showed NEG (N=113), INDF (N=9), or LGD (N=7), respectively (HGD noted on 2nd bx in two).

Conclusions: A 3-yr surveillance strategy for BE-NEG appears appropriate; however, TTE is unpredictable and may be shorter than for BE-LGD.

532 miRNA Expression in Colorectal Carcinoma: Correlation with Microsatellite Instability

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Background: Colorectal carcinoma (CRC) is a common cause of morbidity and mortality in the USA. Several studies have implicated specific microRNAs (miRNA) in colorectal carcinogenesis. A subset of CRC with high levels of microsatellite instability (MSI-H) exhibits distinctive clinicopathologic features, but the correlations between miRNA levels and MSI status have not been studied extensively.

Design: RNA was extracted from paired tumor and histologically normal samples from 27 patients with CRC, 15 microsatellite stable (MSS) and 12 MSI-H cases. Relative expression levels of 6 miRNA (miR-135a, miR-155, miR-31, miR-16, miR-196a and let7a) chosen on the basis of their differential expression in solid tumors and/or CRC were evaluated using stem loop quantitative real-time PCR (qRT-PCR), utilizing 50ng of total RNA per target miRNA. RNA was recovered using the Ambion RecoverAll® kit after manual microdissection of formalin-fixed-paraffin-embedded tissue from unstained glass slides. miRNA levels relative to mean specimen miRNA were calculated by the $2^{-\Delta CT}$ method. Six markers (BAT25, BAT26, BAT40, D2S123, D5S346, D17S250) in the National Cancer Institute panel were used to assess microsatellite instability. The carcinomas were classified as MSI-H if 2 or more markers showed allelic shift and MSS if none showed allelic shift. MSI-H cases were considered due to HNPCC (n=6) if Amsterdam criteria were met or germline mutational analysis was positive for any DNA mismatch repair gene.

Results: Total RNA levels ranged from 802 to 17,496 ng for tumors and from 265 to 4,039 ng for mucosa. miR-31 was over-expressed in tumors relative to non-neoplastic mucosa (13.53 ± 23.1 vs. 1.25 ± 3.0; $p = 0.01$), while miR-196a was under-expressed (0.7 ± 0.5 vs. 1.5 ± 1.1; $p = 0.02$). In MSI-H compared to MSS tumors, lower expression was observed for miR-135 (0.06 ± 0.04 vs. 0.15 ± 0.15; $p = 0.047$) and let-7a (2.7 ± 1.5 vs. 6.0 ± 1.4; $p = 0.005$). Among MSI-H cases, no statistically significant differences were observed in miRNA expression between HNPCC and sporadic MSI-H CRC.

Conclusions: miR-196a was under-expressed, and as has been reported previously, mir-31 was over-expressed in CRC, implicating these miRNA in CRC pathogenesis. Differential miRNA expression in MSI-H and MSS CRC suggests differences at the level of post-transcriptional gene regulation in addition to known differences in gene expression.

533 Distinct Pattern of Acetylation-Modified Histones in the Metaplasia – Dysplasia – Carcinoma Sequence of Gallbladder Carcinoma

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Background: The covalent modification of nucleosomal histones, including posttranslational acetylation, methylation, phosphorylation, and ubiquitination, plays a role in transcriptional regulation and is potentially involved in the propagation of the transcriptional state through cell division. Alterations in histone modification have been linked to deregulated expression of many genes with important roles in cancer development and progression. To determine the histone acetylation modification patterns in the metaplasia-dysplasia-carcinoma sequence of the gallbladder, we examined acetylation of histone H4 at lysine12 (H4K12) and histone H3 at lysine 9 (H3K9) and 18 (H3K18) in gallbladder metaplasia, dysplasia, and carcinoma using immunohistochemistry.

Design: In 2,735 consecutive cholecystectomies performed in the previous 5 years, there were 21 adenocarcinomas, 4 dysplasias, and 21 metaplasias. For each of these cases, representative blocks were chosen and immunohistochemical stains for H4K12, H3K9 and H3K18 were performed. Nuclear staining was considered positive if there was increased intensity in comparison to internal positive controls, normal epithelium and lymphocytes.

Results: The majority of adenocarcinomas were positive for histone 3 acetylation at H3K9 (74%) and H3K18 (80%) and negative for histone 4 acetylation at H4K12 (53%). The patterns of acetylation in adencarcinoma were as follows: All 3 acetylated sites were positive in 5/15, while 4/15 were negative for H4K12 and positive for both H3K9 and H3K18. All other permutations comprised 1 or 2 cases each. Dysplasia had a similar pattern; all four cases were negative for H4K12 and H3K18, and positive for H3K9. The majority of metaplastic epithelium (68%) was negative for all three acetylation sites. However, all 5 intestinal metaplasias showed increases in histone actelyation and composed 5 of the 6 metaplastic cases positive at any acetylation site.

Conclusions: Our results show a distinct pattern of acetylation modification in gallbladder carcinogenesis, indicating this important biological process is involved in the progression of metaplasia to dysplasia. Further, the distinct immunostaining pattern

of acetylated histone 3 and 4 will be helpful in distinguishing metaplastic from dysplastic lesions. It will be significant to further investigate the biologic and pathologic behaviour of gallbladder carcinoma with different histone modification patterns.

534 Immunohistochemical and Molecular Evaluation of Gastric "Hyperplastic" Polyps

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Background: Gastric hyperplastic polyps (HPs) usually develop in association with gastritis and may be multiple. Small gastric HPs are generally considered to be reparative/reactive in nature and often show regenerative epithelial changes, whereas larger lesions occasionally (3%) contain dysplasia. These observations raise the possibility that a subset of gastric HPs represent neoplasms that develop as a result of chronic mucosal injury, although there is very little available data regarding their pathogenesis. The aim of this study was to evaluate the immunohistochemical and molecular features of large (>1 cm) gastric HPs and compare them to the features of small (<1 cm) HPs, adenomas (GA), and mixed hyperplastic/adenomatous polyps (MHAP) in order to better define the pathogenesis of these lesions.

Design: We evaluated 11 large HPs, 10 small HPs, 9 sporadic GAs and 4 MHAPs for the immunohistochemical expression of MLH-1, MSH-2, MSH-6, MGMT, β-catenin, p27 and p16 using standard immunohistochemical techniques. Cases with abnormal nuclear β-catenin staining were assessed for *APC* and β-catenin (*CTNNB1*) mutations. Large HPs, GAs and MHAPs were also evaluated for the presence of *KRAS* and *BRAF* mutations.

Results: Complete loss of MGMT expression was significantly more frequent in large HPs (45%) compared to small HPs and GAs (0% and 0%, respectively, p=0.04). Large HPs also showed loss of p27 (45%) and increased p16 (45%) expression more often than small HPs (10% and 10%, respectively, p>0.05) and GAs [0% (p=0.01) and 33% (p>0.05), respectively]. Nuclear β-catenin staining and corresponding *APC* mutations were present in 44% of GAs, but 0% of large (p=0.03) and 0% of small (p=0.03) HPs. Two (50%) MHAPs showed loss of MGMT staining and 3 (100% of analyzed cases) showed *APC* (2 cases) or β-catenin (1 case) mutations in areas of dysplasia. Loss of MLH-1 staining was noted in only one GA and all polyps showed preserved MSH-2 and MSH-6 expression. *KRAS* or *BRAF* mutations were noted in 18% of large HPs, but 0% of smaller lesions, and 11% of GAs.

Conclusions: Small gastric HPs lack detectable molecular changes and are probably non-neoplastic, reparative lesions, whereas large HPs show loss of MGMT and p27 staining, increased p16 expression, and occasional *KRAS* or *BRAF* mutations. MHAPs similarly show loss of MGMT expression, as well as *APC* or β-catenin mutations limited to areas of dysplasia. These observations suggest that some larger "hyperplastic" polyps of the stomach may represent early neoplasms, similar to colorectal polyps.

535 Gall Bladder Involvement in AIP Is a Primary Manifestation of the Disease

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Background: Autoimmune pancreatitis (AIP) is a steroid responsive chronic mass-forming inflammatory disease of the pancreas. Recent reports suggest that increased numbers of tissue IgG4 plasma cells are a reproducible diagnostic marker for AIP. AIP, primary sclerosing cholangitis (PSC) and biliary obstruction secondary to pancreatic ductal adenocarcinoma (PDAC) are distinct clinical entities, with widely differing outcomes and therapy. However the gall bladder (GB) disease associated with these entities is reported to be histologically similar, reported as diffuse lymphoplasmacytic acalculous cholecystitis (DLACC). Our aim was to identify unique morphological and immunological features, if any, in AIP GBs.

Design: Archival GBs from well characterized individuals with AIP (n=22), PSC (n=13) and PDAC (n=18) were evaluated for mucosal ulceration, location, composition and degree of chronic inflammation, lymphoid nodules, metaplasia and type, fibrosis, presence and degree of phlebitis, and the presence and degree of pseudotumor formation. Pseudotumors were defined as extramural microscopic foci of fibroblasts with embedded lymphocytes and plasma cells. Consecutive AIP and PSC cases were chosen, while the PDAC cases required moderate to marked inflammatory infiltrates. A quantitative immunohistochemical analysis for IgG and IgG4 plasma cells was performed and a IgG4/IgG ratio was derived.

Results: Dense extramural inflammatory infiltrates were almost exclusively seen in AIP (41%), while PSC and PDAC showed similar infiltrates in 0% and 6% respectively (p=0.001). Phlebitis was more frequently noted in AIP cases (p=0.03), although 28% of PDAC GBs also showed phlebitis. Extramural inflammatory pseudotumors were almost exclusively seen in AIP GBs (27%), in comparison to PSC (0%), and PDAC GBs (6%) (p=0.04). AIP GBs showed higher IgG4/IgG ratios in comparison to the other two groups (p=0.0001). Receiver operator characteristics suggest the optimal cut-off IgG4/IgG ratio to distinguish AIP from other diagnoses is 0.47 with specificity of 96% and sensitivity of 83%.

Conclusions: The inflammatory GB involvement seen in AIP is a primary manifestation of this systemic disease and not secondary to biliary obstruction. Morphological features and IgG4/IgG ratios could help distinguish AIP DLACC from its mimics. Evaluation of the GB could help distinguish PSC from the steroid-responsive AIP related cholangiopathy.

536 Thyroid Transcription Factor 1 Is Not Expressed in Gastric Adenocarcinoma: A Study of 220 Cases

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Background: Along with other reports, our previous study (Hum Pathol 2003, 34(6):597-604) showed that thyroid transcription factor 1 (TTF-1) is a valuable marker for the differential diagnosis of pulmonary neoplasms. Thus far, three reports have noted rare partial positive TTF-1 expression in some gastric cancers. However, these were based on single or small series case studies. There has been no systematic study of TTF-1 in gastric cancer. The aim of this study was to assess the prevalence of TTF-1 expression in gastric adenocarcinoma using a large cohort of samples.

Design: We analyzed 220 archival cases of gastric adenocarcinoma of all grades of differentiation by immunohistochemistry. A mouse monoclonal antibody (MAb) (clone 8G7G3/1, 1:200 dilution; Dako, Carpinteria, CA) against the 40-kilodalton nuclear phosphoprotein TTF-1 was used. Lung and thyroid tissues were used as positive controls. Negative control sections were immunostained under the same conditions substituting preabsorbed antisera for the primary antibody. The stained slides were independently reviewed by two pathologists. Expression of TTF-1 was defined as a distinctive nuclear staining. Only unequivocal nuclear staining in the neoplastic cells was considered positive. Cytoplasmic staining was interpreted as negative.

Results: Positive and negative controls displayed appropriate results. No nuclear expression of TTF-1 was observed in any of the 220 cases of gastric adenocarcinoma. Of note, 14 cases displayed remarkable cytoplasmic staining which covered nuclei in two cases.

Conclusions: Our study demonstrated that TTF-1 is not expressed in gastric adenocarcinoma, although nonspecific cytoplasmic staining can be present in a small subset of gastric cancer cases. If metastatic gastric adenocarcinoma is in the differential diagnosis, a positive TTF-1 (nuclear) stain rules out the possibility of a gastric primary.

537 Isolated Asymptomatic Ileitis Does Not Progress to Crohn's Disease Despite Presence of Features of Chronicity in Ileal Biopsies

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Background: It is not uncommon for gastroenterologists to identify isolated ileal abnormalities during routine colonoscopy. The significance of this finding in patients without a prior diagnosis of Crohn's disease (CD) is unknown. Histologic findings of CD may overlap with those of infectious or drug induced ileitis. The aim of our study was to ascertain clinical and/or morphological parameters that are most useful in predicting progression to CD in patients with an isolated ileitis.

Design: Inclusion criteria for the study were: 1) index colonoscopy with ileal biopsies performed at our institution and 2) definite endoscopic and histological evidence of ileal abnormalities upon review. **Exclusion criteria** were: 1) prior history of inflammatory bowel disease; 2) presence of colonic or upper gastrointestinal involvement; 3) patients with follow up less than 3 years; 4) surgical pathology material not available for review. 34 patients met our study criteria. A blinded histopathological examination was performed for a predetermined set of discrete morphological parameters and each case classified into favor CD and favor non-CD groups. Clinical data and outcome were independently retrieved by chart review.

Results: 20/34 patients had colonoscopy for gastrointestinal symptoms while the remaining 14 were asymptomatic (found during screening). Follow up duration ranged from 3-14 years. Overall, 9/17 (53%) patients favored as CD and 3/17 (18%) favored as non-CD, on histological review, progressed to a clinical diagnosis of CD. One or more features of chronicity [crypt disarray or loss, basal lymphoplasmacytosis, pyloric gland metaplasia (PGM)] were present in 10/14 (71%) asymptomatic patients but no granulomas were identified. None of these 14 patients evolved into CD. In contrast, 7/9 (78%) patients with features of chronicity AND presence of symptoms progressed to CD, as did 5/11 (45%) symptomatic patients with no features of chronicity in ileal biopsies. 6/8 (75%) patients in the symptomatic group with PGM progressed to CD while none of the 10 asymptomatic patients with PGM had evolved to CD at last follow up.

Conclusions: Presence of symptoms at the time of colonoscopy is a strong predictor of progression to CD in patients with isolated ileitis. Asymptomatic ileitis does not seem to evolve into CD despite presence of features of chronicity in ileal biopsies.

538 HoxB13 Expression Predicts Increased Disease-Free Survival in Colorectal Carcinoma

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Background: Few prognostic biomarkers are available for colorectal carcinoma (CRC). Our aim was to identify novel reliable prognostic markers for low stage CRC. To this end, we used oligonucleotide microarray expression profile data of CRC and a well annotated clinical database to identify transcripts correlating with clinical progression. Selected markers were validated by analysis of protein expression on tissue microarrays.

Design: HG-U133A microarrays were used to profile 147 primary CRCs. Stage 1 to 3 primary tumors were divided into indolent (no recurrence at 5 yr -53 cases) and aggressive (recurrence or dead of disease {DOD} within 5 yr -16 cases). Criteria used to select candidate genes were >5 fold difference in expression and t-test p< 0.01 between indolent and aggressive primary CRC groups. Protein expression was investigated by IHC using 3 tissue microarrays containing 225 cases of primary and metastatic CRC. Staining intensity (0 to 3) and percent of tumor labeling were recorded. Final intensity was computed as average intensity of individual cores weighted by percentage of involvement. Cases were divided into high and low expressors, and Kaplan-Meier curves were computed to assess correlation with recurrence and survival.

Results: One of the two transcripts identified, HoxB13 was overexpressed by 6.1 folds in indolent versus aggressive CRC (p=0.001). HoxB13 appears to inhibit the growth of CRC cells by interfering with the Wnt signaling pathway and was therefore selected for further analysis at protein expression level. Out of 151 cases of primary CRC, 32 showed strong and 119 showed weak or no staining for HoxB13. There were significant differences in survival and disease free interval between high and low expressors (164 vs 86 mos, p=0.03 and 189 vs 97 mos, p=0.04, respectively). When only stage 1 to 3 CRCs were analyzed, there were no recurrences or DOD events in the high expressor group at a median follow-up of 58 mos (24-112 mos) whereas the low expressors had a survival rate of 83% and disease-free rate of 72% at 5 years.
Conclusions: In our study, expression of HoxB13 protein stratified low stage CRC into two groups with different clinical outcomes. These results strongly suggest that HoxB13 might serve as a prognostic biomarker in low stage CRC. Further validation studies are necessary to confirm the clinical relevance of HoxB13 expression and its role in colonic carcinogenesis.

539 Clinicopathologic Analysis of Large and Giant Condyloma Accuminata of the Anus

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Background: It is well established that anal condylomas have a potential for malignant transformation. However, the relationship of the size with malignant potential has not been well documented. In fact, whether a giant condyloma (Lowenstein-Buschke) represents a very large version of ordinary condyloma, or a form of verrucous carcinoma, and the incidence of conventional malignant change (CIS or ordinary invasive carcinoma) in these large tumors have not been adequately studied.
Design: In the authors' institutional files, 80 cases of anal condylomas that measured > 2 cms were investigated. The cases were classified based on their size as medium-large (2-5 cm, n = 61), very large (5-10 cm, n = 13) and giant (>10 cm, n = 6).
Results: The vast majority of the patients were males (M:F = 8:1). The proportion of males appeared to be even higher in smaller lesions. Overall mean age was 38 years. The giant (>10 cm) condylomas occurred in patients a decade older (mean 46 *vs* 37, p = 0.09). The overall incidence of conventional high-grade dysplasia was 10%, all of which occured within the 2-5cm category. Full-blown CIS was detected in 4% of cases, also all within the 2-5cm category. Invasive squamous cell CA was found in 15% of the cases overall. There was a statistically significant trend between incidence of invasion and increasing size: 10% for 2-5cm group, 27% for >5cm cases, and 50% in those >10 cm (p = 0.02).
Conclusions: It is advisable that large (>2 cm) anal condylomas further categorized based on their size, and that the term giant reserved for those that are >10 cm, which seem to occur in patients a decade older, suggesting a slow growing process. The overall incidence of invasive carcinoma in large anal condylomas in this study was 15%, and there was a trend for increasing incidence of invasion with size.

Table 1.

Size	Case Number	Average Age	M:F	HG Dysplasia (%)	CIS (%)	Invasion (%)	Known HIV+ (%)
2-5 cm	61	38	14:1	8 (13)	3 (4.9)	6 (10)	13 (21)
5-10 cm	13	37	5.5:1	0	0	3 (27)	3 (23)
> 10 cm	6	46	2:1	0	0	3 (50)	0
Total	80	38	8:1	8	3 (3.75)	12 (15)	16 (20)

540 DNA Repair Proteins MLH1, MSH2, MSH6, and MGMT in 495 Gastric and Esophageal Adenocarcinomas

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Background: Defective DNA mismatch repair (MMR) is thought to promote tumorigenesis by accelerating the accumulation of mutations in oncogenes and gene suppressor tumors.
Design: We investigated the expression of the DNA repair enzyme that protects against alkylating mutagens, O6 methylguanine DNA methyltransferase (MGMT), and the MMR enzymes, MLH1, MSH2 and MSH6, in 480 gastric cancer specimens and 15 esophageal adenocarcinomas by immunohistochemical analysis using tissue microarrays (TMA).
Results: Loss of expression of DNA repair enzymes MLH1, MSH2, MSH6 and MGMT in gastric adenocarcinomas was observed in 2.2%, 0.6%, 15.6% and 4.8%, respectively. However, esophageal adenocarcinomas showed a very low loss of those proteins, varying from 0% for MLH1 and MSH2 and 3.6% for MSH6 and MGMT. Evaluation according to histological type of gastric cancer demonstrated a significant correlation between loss of MSH6 and Laurén intestinal-type adenocarcinomas (p=0.02). Besides, negativity of MSH6 was related with no regional lymph node metastases in gastric adenocarcinomas (p=0.02).
Conclusions: These findings demonstrate that defective DNA mismatch repair enzymes are associated with gastric carcinogenesis but do not play a role in the pathogenesis of esophageal adenocarcinomas. Loss of MSH6 is associated with a better prognosis in gastric adenocarcinomas.

541 Up-Regulation of the HIF-1 Transcriptional Pathway in Colorectal Carcinomas

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Background: The aim of the study was to identify the impact on prognosis of HIF-1 genetic program in colorectal carcinomas (CRCs) and to develop an experimental procedure that would allow a reliable quantitative gene expression analysis in formalin-fixed and paraffin-embedded (FFPE) tissue.
Design: The expression of HIF-1α and thirteen HIF-1 target genes was quantified by real time PCR in 78 FFPE tissue specimens of CRCs and in 8 samples of normal colorectal mucosa. The expression of HIF-1a protein was also evaluated by immunohistochemistry in all tumor samples.
Results: A general up-regulation of HIF-1α and its target genes was observed in cancers compared with normal samples. Overexpression of HIF-1α protein was found in 57 of 74 (77%) tumors. Although poorly differentiated CRCs with high mitotic rate were found to be positively associated with HIF-1α up-regulation, no association between high levels of HIF-1α expression and poor overall survival was detected. However, CRCs exhibiting a highly aggressive behavior show a significant up-regulation of HIF-1 responsive genes, suggesting that the consequences of HIF-1 α overexpression are strongly dependent on the cellular context. The multivariate analysis showed that advanced stage, presence of lymph node metastases and high levels of TGFα had an independent effect on survival (p<0.006; p<0.01; p<0.0006).
Conclusions: These findings suggest an up-regulation of the HIF-1 transcriptional pathway in CRCs and confirm *in vivo* its association with tumor growth and aggressiveness. A quantitative real time PCR assay can be used as a sensitive diagnostic technology to measure mRNA from archival tissue.

542 Prevalence of Helicobacter Infection in Biopsies Taken from the Gastroesophageal Junction ***Only*** for the Evaluation of Reflux

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Background: In daily practice, we have come across a subset of patients with biopsies taken *only* from the gastroesophageal junction (GEJ) for evaluation of reflux, that showed Helicobacter pylori (HP) carditis- an unexpected finding. In this study, we aimed to determine the prevalence of HP infection and elucidate the histological and clinical features from these GEJ only biopsies.
Design: Consecutive patients who underwent GEJ only biopsies for reflux were studied. Biopsies containing both squamous and cardiac mucosa were included with Barrett's cases being excluded. GEJ only biopsies with active esophagitis (study group) was compared to the following: GEJ biopsies from patients with simultaneous gastric biopsies with HP positive gastritis (HpG), chronic inactive HP negative gastritis (CIG), chemical gastropathy and normal GEJ only biopsies (Control). All the GEJ biopsies were evaluated using H&E stain and HP immunostain.
Results: 198 patients were included, with 114 patients in the study group (mean 56 years, M:F-1:1.36), 54 in the HpG group, and 10 each for the CIG, chemical and normal control groups. HP was detected in 22/114 (19.3%) GEJ biopsies from the study group, 43 (80%) from HpG group. HP was not detected (0%) in the CIG, chemical and normal control groups. The HP positive GEJ biopsies showed mild to moderate carditis with mild to moderate plasmacytosis (22/22), and active inflammation (17/22). 62 cases (54%) from the study group showed histologic features of reflux, with 7/62 cases (11%) positive for HP. In the remaining 52 patients without histologic evidence of reflux, 15/52 (29%) were positive for HP. All 10 cases of erosive esophagitis with marked carditis were negative for HP and were likely acid related. The endoscopic findings for patients with HP infection in the study group consisted of esophagitis, suspicious for Barrett's tongue, hiatal hernia, stricture, irregular Z-line, Schatzki ring, nodule and normal. All the patients from the study group had normal appearing stomach that was *not* biopsied.
Conclusions: 19% of GEJ only biopsies taken for reflux showing active carditis were positive for Helicobacter pylori. These patients had reflux symptoms with endoscopically normal looking stomach. Our results stresses the importance of taking simultaneous gastric biopsies, in addition to GEJ biopsies, and also the need for vigilance in pathologists when confronted with a GEJ biopsy with increased plasma cells and active inflammation to order a HP immunostain.

543 Clinicopathologic Study of Gastrointestinal Stromal Tumors (GIST) from a Single Institution with Emphasis on Prognostic Stratification Based on the National Institute of Health (NIH) Consensus Criteria

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Background: GISTs are the most common mesenchymal tumors of the GI tract. To date, there are no clear criteria in assessing malignancy in GIST. The aim of this study is to review the clinicopathologic features of GIST and assess the prognostic value of stratification based on the NIH criteria (tumor size and mitotic activity) in a retrospective analysis of cases from a single institution.
Design: All patients from 1989-2007 diagnosed with GIST (including c-kit positive and c-kit negative) were selected from our surgical pathology computerized database. The patients' age, sex and tumor location, including presence of synchronous tumors or metastases were recorded. Follow-up information was reviewed to identify patients with recurrence and/or metastases.
Results: A total of 79 GIST cases were identified, these included 44 males and 35 females with an age range of 35-84 years (mean 73years). The tumor size ranged from 0.4cm to 30cms. A mean follow up of 47.3months was available in 71 patients. The location of GIST included stomach (40, 50.6%), esophagus (2, 2.5%), small intestine (23, 29.11%), colon/rectum (8, 10.1%), abdomen/pelvis (6, 7.6%). Based on the NIH prognostic criteria, the tumor categories included: high risk (HR) (37.9%), intermediate risk (IR) (12.6%), low risk (LR) (27.8%) and very low risk (VLR) (21.5%). Synchronous non-GIST tumors were present in 21 (3-IR, 5-LR, 13-VLR) cases. Metastases were present at the time of diagnoses in 16 patients, all HR GIST. Seven of these 16 patients developed metastases on follow up. Nine other patients with HR GIST free of metastases at time of presentation had recurrence or distant metastases. The most common site for metastases included peritoneum (10) followed by liver (8). Two HR patients died due

to progressive disease while 2 LR patients died due to unrelated causes. One patient with LR developed abdominal LN metastases 61 months after diagnosis. None of the IR or VLR developed recurrence or metastases.
Conclusions: Our results validate the prognostic utility of the NIH consensus criteria in predicting GIST behavior. Metastasis, although rarely, can occur in LR GIST. The clinical behavior of GIST can be predicted with reasonable accuracy using the combination of tumor size and mitotic activity.

544 Mucosal Schwann Cell Hamartoma: Clinicopathologic Study of 22 Neural Colorectal Polyps Distinct from Neurofibromas and Mucosal Neuromas

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Background: Colorectal intramucosal neural proliferations that lack ganglion cells have been variously referred to as "neuromas" or "neurofibromas." However, these lesions have not been systematically examined, and whether they are associated with type 1 neurofibromatosis (NF1) is unknown. The aim of this study was to evaluate the clinicopathologic and immunohistochemical features of these lesions, in comparison to neurofibromas from known NF1 pts.
Design: Polyps were retrieved by searching for the following terms: neural, neuroma, neurofibroma, S-100. After ganglioneuromas and perineuriomas were excluded, morphologically similar lesions from 22 pts (mean age, 63 yrs; range, 46-88; M/F ratio, 8/14) were identified. Clinical and endoscopic data were obtained, and immunohistochemistry for S-100, GFAP, neurofilament protein (NFP), EMA, claudin-1, CD34, SMA, and c-kit was performed. The findings were compared to those in biopsies of 5 submucosal neurofibromas from NF1 pts.
Results: All 22 polyps were sessile, ranging from 1-6 mm (mean, 2.5 mm). Most arose in the distal colon (12 rectosigmoid; 6 descending; 2 transverse; 2 ascending), incidentally found at screening colonoscopy. After a mean follow-up of 4 yrs (range, 3 mos-16 yrs), none of the pts developed other neural polyps and none had NF1. Histologically, the polyps contained a diffuse intramucosal proliferation of uniform bland spindle cells with elongated, tapering nuclei and indistinct cell borders, in a dense collagenous stroma. No nuclear atypia, pleomorphism, mitotic activity, or associated ganglion cells were observed. All showed strong staining for S-100 in essentially 100% of cells. NFP highlighted rare axons in 6 lesions. All other markers were negative. The 5 neurofibromas showed similar histologic features, but less extensive staining for S-100; all contained scattered NFP-positive axons.
Conclusions: Colorectal polyps consisting of pure Schwann cell proliferations limited to the mucosa are not associated with NF1. Distinguishing these lesions from NF1-associated neurofibromas is difficult based on biopsy findings alone; the presence of an underlying submucosal nodule or mass should be excluded endoscopically. In contrast to mucosal neuromas associated with MEN-2B (which contain numerous axons), axons are usually absent in these lesions. Although their nature is uncertain, we propose the interim designation "mucosal Schwann cell hamartoma" to avoid confusion with the neural lesions that have significant associations with inherited syndromes.

545 Proposed Modifications in the Protocol for Rectal Adenocarcinomas Resected after Preoperative Radiochemotherapy

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Background: Patients with locally advanced rectal adenocarcinomas (T3/T4 and/or N1) are given preoperative radiochemotherapy (RTC) before resection to reduce local recurrence and improve survival. At resection, the down-staged tumors are sufficiently altered that usual CAP/ADASP colorectal carcinoma pathology reporting protocols warrant modifications. Novel modifications are proposed that incorporate post-RTC asymmetrical tumor configuration, buried tumor foci, nodal shrinkage, attempted sentinel node mapping and level of downstaging.
Design: Between 2005 and June of 2007, in addition to the recommended CAP/ADASP protocol, we applied an internally developed novel sampling protocol to 16 post-RTC resected rectal adenocarcinoma cases. 3/16 cases also had peritumoral dye injection for sentinel node mapping. Clock-face mapping up to 4 cm (1 cm increments) in all directions around residual tumor was performed with comparisons of gross and microscopic residual tumor configuration and size, mean number and size of lymph nodes, tumor shrinkage and level of downstaging (pre and post RTC stages).
Results: 10/16 (62%) cases were down-staged with 1 showing no residual tumor. In 13/16 (81%) cases the microscopic tumor was larger but in all residual 15 (100%) cases, the configuration was skewed asymmetrically away (range 0.5-4 cm, mean 2.1 cm) from the gross tumor with buried tumor foci under preserved mucosa. Sentinel node mapping was unsuccessful in the 3 attempted cases. The mean number of nodes was 10 and the mean size was 3.3 mm. Adherence to the CAP/ADASP protocols which recommends to optimally take five sections from the center of the lesion does not allow for complete recovery of tumor foci post RTC. Despite intensive searching, the mean number of lymph nodes retrieved in our study fell below the CAP/ADASP recommended number of 12-15.
Conclusions: Post-RTC tumor foci are skewed asymmetrically away from apparent epicenter and require a "clock-face" sampling of at least 4 cm in all directions to recover buried tumor foci. Sentinel node mapping is unsuccessful likely due to fibrous obliteration of lymphatic channels and is not beneficial in post-RTC resections. There is drastic nodal shrinkage necessitating re-appraisal of acceptable number of obtained nodes. Pre-RTC tumor data needs to be incorporated in the report to accurately reflect down-staging and tumor alterations.

546 Specificity of Chronic Radiation Colitis in Endoscopic Mucosal Biopsies

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Background: Chronic radiation colitis is clinically diagnosed when diarrhea and/or rectal bleeding is associated with endoscopically visible colitis and prior history of local radiation exposure. Histologically, the endoscopic colonic biopsies do not include the submucosa which could provide histological signs of radiation such as stromal cell atypia and obliterative arteritis. The mucosal changes of chronic radiation colitis are distinctive but mimicked by ischemic colitis, ulcerative colitis, angiodysplasia and portal hypertensive colopathy. This study is undertaken to evaluate specificity of mucosal changes in chronic radiation colitis.
Design: 28 adults (16 F and 12 M, age 42-79, mean 64) with history of radiation (for uterine cervical, endometrial or prostate carcinomas) and clinical, endoscopic and colonoscopic biopsy diagnosis of chronic radiation colitis were selected from our files. Their routine HE stained sections were reviewed and compared with colonoscopic mucosal biopsy controls consisting of 20 cases each of ischemic and ulcerative colitis, 3 cases of portal hypertensive colopathy and 4 cases of angiodysplasia. The following were analyzed morphometrically: mean capillary diameter, mean thickness of pericapillary hyaline and mean percentage of plasma cells in unit areas (3000 square μm) of the lamina propria. The results were analyzed for statistical significance.
Results: The mean plasmacytosis (72%) and mean thickness of pericapillary hyaline (9 μm) was greater in chronic radiation colitis than all other assessed conditions ($p=<0.05$). In ischemic colitis, there was generalized stromal hyalinization but mean pericapillary hyaline band was only 3 μm. The capillary ectasia (mean capillary diameter 71 μm) seen in chronic radiation colitis, however, is not significantly different from that seen in angiodysplasia (75 μm) and portal hypertensive colopathy (72 μm).
Conclusions: While ectatic capillaries are also seen in angiodysplasia and portal hypertensive colopathy, thickened pericapillary hyalinized band and selective lamina propria plasmacytosis are characteristically and uniquely seen in chronic radiation colitis and not in disorders considered in the differential diagnoses. With history of radiation, the triad of ectatic capillaries, pericapillary hyalinization and plasmacytosis is conclusive of chronic radiation colitis in colonoscopic mucosal biopsies.

547 Differences in Pediatric and Adult Granulomatous Gastritis: A Clinicopathological Analysis of 40 Patients

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Background: Granulomatous gastritis (GG) is seen in less than 0.5% of gastric biopsies. Gastric granulomas are found in systemic granulomatous diseases and less commonly in infectious diseases. The purpose of this report is to analyze the clinical and pathological features associated with GG in adult and pediatric patients.
Design: Retrospective analysis identified 21 children and 19 adults with GG on biopsy during the period January 2000 to August 2007. Clinical information recorded for each patient included age, sex, and final clinical diagnosis. Slides were stained with hematoxylin and eosin, toluidine blue, Kinyoun' method for acid fast organimss and Grocott's method for fungal organisms. Biopsies were evaluated for the presence of active and chronic gastritis, diffuse or multifocal inflammation, *Helicobacter pylori* organisms, acid-fast bacilli, and fungal organisms. The location of the granulomas in the stomach.was recorded.
Results: Children ranged in age from 5-17 (mean 12.4 years) with a M:F ratio of 2.5:1. Final clinical diagnoses in pediatric patients included Crohn's disease (80.9%), ulcerative colitis (9.5%), gastritis (4.8%), and gastroesophageal reflux disease (4.8%). Adults ranged in age from 25-86 years (mean 54.2 years) with a M:F ratio of 1.7:1. Final clinical diagnoses in the adult patients included sarcoidosis (26.3%), gastrointestinal bleeding with anemia (21%), gastritis (21.1%), gastroesophageal reflux disease (15.8%), Barrett's esophagus (5.2%), Crohn's disease (5.2%), erosive gastritis and ulcer (10.5%), and incidental finding during screening examination (5.2%). In both groups, granulomas were most often located in the antrum (children 90.5%, adults 94.7%). Active chronic gastritis was seen in 38.1% of children; the one child with *Helicobacter pylori* infection also had Crohn's disease. Active chronic gastritis was seen in 47.4% of adults; three with *Helicobacter pylori*. In children, GG with Crohn's disease presented as diffuse chronic gastritris with or without active inflammation equally (47.1% each) and mulifocal gastritis in 5.9%. In adults, GG was more evenly distributed among a number of disease entities, being slightly more common in sarcoidosis (26.3%).
Conclusions: In children, GG is most commonly seen in Crohn's disease. In adults, GG is associated with many diseases, including sarcoidosis.

548 Immunohistochemical Assessment of Transforming Growth Factor-Beta in the Pathogenesis of Collagenous Colitis

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Background: Collagenous colitis (CC) is a cause of chronic diarrhea with an unknown pathogenesis that is histologically characterized by increased subepithelial collagen deposition. Myofibroblasts in the liver have been described as effector cells in the pathogenesis of cirrhosis, and more recently have also been implicated in the pathogenesis of fibrosis in chronic pancreatitis. Studies have shown a strong correlation between hepatic fibrosis and the presence of inflammatory cytokine transforming growth factor-beta (TGF-B). The purpose of our study was to evaluate colonic lamina propria myofibroblasts in the pathogenesis of CC and to assess the role of TGF-B in this process.
Design: The study included 36 cases, 20 with clinical symptoms of CC and histologic confirmation of an increase in subepithelial collagen table thickness, and 16 cases with symptoms similar to CC, but biopsies either showing chronic inflammation with a minimally thickened collagen table, or completely unremarkable biopsies.

The collagen table thickness was measured from below the basement membrane to the bottom of the table and the thickest area recorded. Antibody to smooth muscle actin (SMA) was used to identify lamina propria myofibroblasts. Three pathologists independently evaluated the proportion of lamina propria myofibroblasts and correlated it with the collagen thickness. These biopsies were then stained with antibody to TGF-B and the intensity and percentage of staining of lamina propria myofibroblasts were independently evaluated.

Results: There was a significant increase in the number of myofibroblasts in the lamina propria in CC with a linear correlation between the number of myofibroblasts, identified by SMA, and collagen layer thickness in CC patients compared to those with chronic inflammation ($p=0.06$) or unremarkable biopsies ($p<0.0001$). Staining with TGF-B revealed positivity in a smaller percentage of myofibroblasts compared to SMA, however the staining intensity with TGF-B was significantly higher in biopsies with collagen table thickness > 15 microns.

Conclusions: Colonic lamina propria myofibroblasts may play an integral role in the pathogenesis of CC. We speculate that TGF-B is a signaling molecule involved in the activation of lamina propria myofibrolasts and may therefore serve a potential target for further investigations and drug discovery.

549 High-Grade Squamous Dysplasia with Partial Mucosal Thickness Involvement (PTI): A Common Precursor Lesion of Invasive Squamous Cell Carcinoma of the Esophagus

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Background: The diagnosis of squamous dysplasia in mucosal biopsies of the esophagus is challenging since the histologic criteria applied to these lesions in other sites are not entirely applicable to this organ. It is our impression that the traditional grading criteria based on the proportion of the mucosa replaced by dysplastic cells may underestimate high-grade (HG) lesions with PTI. This impression is based on the observation that invasive carcinoma may derive directly from HG dysplastic lesions with PTI. The goal of this study is to examine the frequency and significance of HG dysplasia with PTI and to define the diagnostic criteria for their identification on biopsies.

Design: 20 esophagectomy specimens were studied, 17 from patients with invasive squamous cell carcinoma and 3 with non-neoplastic conditions. Cases with dysplasia were evaluated for the following features: distribution of dysplastic foci in relation to the invasive tumor and to each other, cytologic characteristics of the dysplastic cells and proportion of transmucosal replacement by dysplastic cells. Full thickness involvement (FTI) was defined as replacement of >1/2 of the epithelial thickness and PTI as replacement of <1/2. Cells with HG cytology were identified by high N/C ratio, pleomorphic nuclei with chromatin clearing and prominent nucleoli. Immunohistochemical reactions for Ki67 (clone MM1, Visionbiosystems), p53 (clone BP53-11, Ventana) and p16 (clone 16P04/JC2, Cell Marque) were performed on selected slides from all neoplastic and nonneoplastic cases.

Results: Although dysplastic lesions were predominantly located near invasive tumor, foci of dysplasia were frequently seen >1 cm away from the tumor. In all specimens, patchy rather than diffuse involvement was observed. In 2 specimens FTI was the sole type of dysplasia. PTI was present in 15 cases, 10 of which had concomitant FTI. Invasive carcinoma was seen "dropping off" from dysplastic foci with PTI in 3 cases. With the exception of one case, p53 intensely labeled all cells with HG cytology, but not the esophagi with non-neoplastic diseases; Ki67, although useful, was less reliable than p53. Staining for p16 was not helpful.

Conclusions: HG dysplasia with PTI is commonly present in the esophagi that harbor an invasive carcinoma and can be identified by cytologic criteria and by intense immunoreactivity of the dysplastic cells for p53.

550 Differential Expression of NF-kappa B and Bcl-3 in Colorectal Carcinoma and Paired Normal Colorectal Mucosa

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Background: NF-kappa B is a predominantly anti-apoptotic set of transcription factors that is comprised of homo- or hetero- dimers of five subunits. NF-kappa B is classically repressed by I-kappa B proteins and activated when I-kappa B proteins are ubiquitinated and degraded, releasing NF-kappa B. The basis for abnormal NF-kappa B activity in solid tumors is unknown. Our group has recently described overexpression of an atypical I-kappa B-like molecule, Bcl-3, in hepatocellular carcinoma. To date, there has been no study of Bcl-3 in colorectal cancer. We sought to assess Bcl-3 and the three NF-kappa B subunits most associated with Bcl-3 overactivity in colorectal cancer and its paired normal colonic mucosa.

Design: Tissue microarrays were constructed from primary colorectal carcinoma, normal colorectal mucosa, and metastatic carcinoma to liver and lymph nodes from 24 patients. Each tissue type is represented in triplicate. Immunohistochemical staining for NF-kappa B family proteins (p50, p52, and p65), Bcl-3, and Caspase3 was performed with DAB detection. Nuclear and cytoplasmic staining intensity (0 to 3+) and percentage (0-100%) of positively staining cells of interest were scored, and the product was calculated (0 to 300). Means of the products were calculated for each patient, and the Wilcoxon rank sum test was used to determine the statistical significance of the difference in the means between the primary and the normal mucosa.

Results:

Nuclear Staining

Protein	Product Mean - Primary	Product Mean - Normal	P Value
p50	9	12	NS
p52	0	0	NS
p65	82	44	<0.05
Bcl-3	151	159	NS
Caspase-3	96	113	NS

Cytoplasmic Staining

Protein	Product Mean - Primary	Product Mean - Normal	P Value
p50	187	108	<0.01
p52	251	41	<0.01
p65	257	116	<0.01
Bcl-3	186	102	<0.05
Caspase-3	189	204	NS

Conclusions: We have shown a statistically significant increase in expression of the NF-kappa B proteins p50, p52, and p65 within the cytoplasm of tumor cells from colorectal carcinoma primaries compared to normal mucosa from the same patient. Additionally, a statistically significant increase in p65 nuclear staining in tumor cells compared to normal mucosal epithelium is noted. This may represent p65 protein activation, as measured by nuclear translocation, and is a potential target for further research. Bcl-3 expression was also shown to be increased within the cytoplasm of primary tumor cells. This study was supported by the UNC GI Cancer SPORE.

551 Fibroblastic Polyps of the Colon and Colonic Perineuriomas: Two Names for a Single Entity

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Background: Fibroblastic polyps and perineuriomas of the colon are unusual mucosal lesions with identical clinical and histologic features and apparent different immunohistochemical and ultrastructural characteristics. Immunohistochemical distinction however, was solely based on results obtained with epithelial membrane antigen (EMA), an antibody whose reactivity on perineuriomas is difficult to demonstrate. Likewise, accurate ultrastructural diagnosis may be flawed by sampling error, preservation artifacts or paucity of specific diagnostic features. In a recent short communication it was suggested that both lesions may represent a single entity. To further evaluate this hypothesis we studied by immunohistochemistry and electron microscopy a series of 26 polyps with clinical and histologic characteristics of fibroblastic polyps/perineuriomas.

Design: Twenty-six colorectal polyps with clinical and histological features of colonic fibroblastic polyps/perineuriomas including 10 cases previously reported as fibroblastic polyps (Ref.: *Histopathology*. 2006;48:431-437) were stained immunohistochemically for 4 markers of perineurial differentiation: claudin-1, glut-1, collagen type IV and EMA (the latter performed using an extended protocol for antigen retrieval and a kit for signal amplification). Electron microscopy was carried out in 4 cases.

Results: EMA and claudin-1 stained 24 of 26 (92%) polyps whereas GLUT-1 and collagen IV were expressed by all the lesions. EMA staining was mostly focal and weak while the other markers exhibited a diffuse and strong signal. Ultrastructurally, tumor cells showed features supportive of perineurial differentiation including long, very thin cytoplasmic processes, discontinuous external lamina, pinocytotic vesicles and rare tight junctions.

Conclusions: Our findings support the hypothesis that fibroblastic polyps and perineuriomas of the colon are identical structures that represent the same lesional process. Accordingly and based on the perineurial nature of the tumor cells we suggest reclassifying fibroblastic polyps of the colon as perineuriomas.

552 Expression of MUC-6, Gastric (Sox-2) and Intestinal (Cdx-2) Transcription Factors in Long Segment Barrett's Esophagus: A Complex Interplay at the Neo-Squamocolumnar Junction

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Background: Barrett's esophagus (BE) is believed to arise on a background of acquired gastric cardia type mucosa, although direct evidence is lacking. The metaplastic transformation in BE is mediated through intestinal transcription factor Cdx-2. Although considerable controversy exists regarding the presence of gastric cardia type mucosa around the gastroesophageal junction, the characteristics of the proximal advancing edge of BE have not been studied in detail. In this study, we analyzed the expression of gastric (Sox-2) and intestinal (Cdx-2) transcription factors and pyloric–gland mucin (MUC-6), in the most proximal biopsies from a series of patients with well documented long segment BE.

Design: Inclusion criteria for the present study were: 1) definite endoscopic evidence of more than 3cm of columnar mucosa in the distal esophagus; 2) presence of goblet cells on biopsy; 3) sequential biopsies from every 1-2cm available for evaluation; 4) no evidence of dysplasia in any of the biopsies. The most proximal biopsies from the neo-squamocolumnar junction from 39 patients, thus formed the study group. All cases were stained immunohistochemically with Sox-2, MUC-6 and Cdx-2 using commercially available antibodies. Staining was evaluated in surface and crypt epithelium and in basal cardia type mucous glands when present.

Results: Immunohistochemical expression of Sox-2 was present in the surface epithelium in 5/39, in the crypt epithelium in 15/39 and in basal cardia type mucous glands in 31/39 cases. In contrast, Cdx-2 staining was observed in the surface epithelium in 35/39 cases, in crypt epithelium in 38/39 cases and focal weak staining was observed in basal mucous glands in only 8/39 cases. MUC-6 positivity was present in 38/39 cases and was only seen in basal cardia type glands or in the lower half of the metaplastic crypts. No staining was observed in surface foveolar type epithelium. The only case completely negative for MUC-6 was also negative for Sox-2.

Conclusions: Sox-2 and MUC6 are expressed in cardia type mucous glands at the "advancing edge", in a majority of patients with long segment BE. Reduced Sox-2 expression in upper crypt and surface epithelium coincides with increasing expression of Cdx-2. Our findings support to the contention that BE arises in a background of acquired gastric cardia type mucosa.

553 DNA Ploidy Abnormalities in Non-Goblet and Goblet Columnar Epithelium in Barrett's Esophagus

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Background: Previous studies have shown that non-goblet (NG) "cardia type" epithelium in Barrett's esophagus (BE) is physiologically intestinalized, and hyperproliferative. The relationship of density of goblet cells (GC) to risk of malignancy in BE is unknown. Some BE-related adenocarcinomas develop in mucosa devoid of GC. The aim of this study was to evaluate DNA content, by image cytometry, using high fidelity histograms, in NG and GC epithelium in patients with columnar metaplasia of the distal esophagus (CMDE).

Design: Sixty-seven routinely processed mucosal biopsies from 11 patients with CMDE without GC (Group1), 16 patients with CMDE with low-density GC (Group 2), and 12 patients with CMDE with high-density GC (Group 3) were evaluated by image cytometry. In Groups 2 and 3, areas either with or without GC were also evaluated separately. High-fidelity DNA histograms were analyzed for peak DNA index, DNA heterogeneity index (HI; representing groups of cells with different DNA content), and percentage of cells with DNA exceeding 5N (5N-ER). These parameters were compared between the three groups of patients, and also within separate samples in Groups 2 and 3.

Results: The mean DI (1.08, 1.14, 1.18), frequency of aneuploidy (55%, 56%, 67%), and 5N-ER (0.36, 0.2, 0.2) in Groups 1, 2 and 3 respectively, were statistically similar (P>0.05). When NG and GC epithelia were compared within each of the Group 2 and 3 patients separately, the DI (1.07) and HI (17.3) values, but not the 5N-ER, in NG epithelium were significantly lower (P<0.05) than GC epithelium only in Group 3 patients (DI=1.18, HI = 19.7). Biopsy samples without GC compared to those with GC, in Group 2 patients (with low-density GC), did not differ significantly from each other.

Conclusions: Non-goblet CMDE shows DNA content abnormalities similar to those of patients who fulfill the definition of BE (which is defined by the presence of GC). DNA content abnormalities are more severe in areas of GC compared to the background non-GC epithelium, only in patients with high-density GC in their CMDE, but otherwise, are unrelated to the density of GC. This data raises doubt regarding the current ACG definition of BE, which requires GC to be present in order to establish the diagnosis, and supports the concept that NG CMDE has malignant potential.

554 Perineural Invasion in Colorectal Cancer: A Reliable and Independent Prognostic Marker

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Background: Studies have demonstrated perineural invasion (PNI) to be a poor prognostic factor in colorectal carcinoma (CRC). However, these studies have examined several histologic markers and have not focused on perineural invasion alone. Previous studies have shown high interobserver variability in the diagnosis of lymphovascular invasion (LVI). PNI may prove to be a more reliable and consistent marker of poor patient outcome.

Design: One hundred fifty-six cases of AJCC stage II-IV CRC from 1995 to 2005 were selected from the pathology archives. Mucinous, medullary, and signet-ring histology were excluded. Cases were examined for the presence of PNI. Correlation with tumor stage, grade, LVI, lymph node metastases, and patient outcome was performed.

Results: Twenty-seven (17%) of 156 cases demonstrated positive PNI (Figure 1, Table 1). Patient age, sex, and treatment were not significantly different between the two groups. LVI was present in 93% of cases with PNI versus 43% of cases without PNI. Lymph node metastases were present in 78% (+PNI) versus 40% (-PNI). Forty-one percent of cases with PNI were grade 3 tumors versus 15% (-PNI). Fifty-six percent of +PNI cases were AJCC Stage 4 versus 24% (-PNI). Mean survival of patients with PNI was 19 versus 30 months in patients without PNI (Table 2).

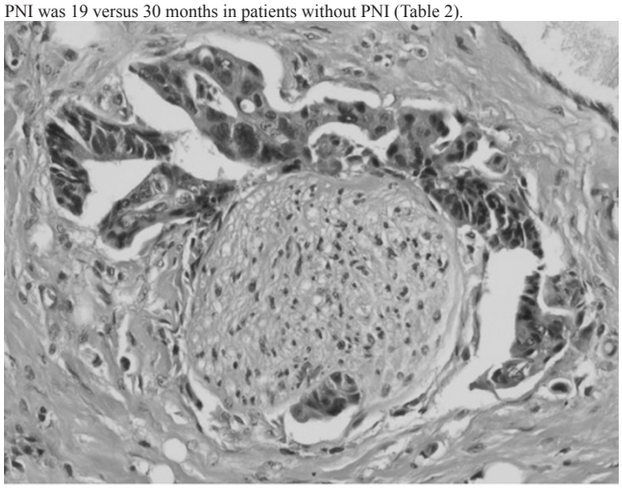

Figure 1: Positive perineural invasion. H&E, 200x.

Table 1: Patient characteristics (n=156)

	+ PNI	- PNI
	27 (17%)	129 (82.7%)
Age (average)	35-86 (60)	27-89 (62)
Grade 1	0	9 (7%)
Grade 2	16 (59%)	101 (78.3%)
Grade 3†	11 (41%)	19 (15%)
Stage II‡	0	56 (43%)
Stage III	12 (44%)	42 (33%)
Stage IV†	15 (56%)	31 (24%)
LVI present†	25 (93%)	55 (43%)
LN metastases†	21 (78%)	52 (40%)

†p<0.05; ‡p<0.005

Table 2: Patient outcomes

	+ PNI		- PNI	
Cases (n=156)	27	F/U (avg)	129	F/U (avg)
NED	7 (26%)	9-114 (58)	82 (64%)	1-135 (33)
Mean survival (months)†	19		30	
AWD	11 (41%)	6-85 (30)	22 (17%)	1-46 (19)
DOD	9 (33%)	2-52 (19)	20 (16%)	1-74 (30)

†p<0.005; NED: no evidence of disease; AWD: alive with disease; DOD: died of disease; F/U: follow-up, months

Conclusions: PNI in CRC is significantly associated with higher grade and stage of disease and poor patient outcome. As pathologist agreement on the diagnosis of LVI is poor, the inclusion of PNI in CRC staging summaries may be warranted.

555 Phospho-A20 Immunostaining Can Predict Patient Responsiveness to Anti-TNF Therapy

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Background: Anti-TNF agents (Remicade) are efficacious in Crohn's Disease (CD), however, only 60-70% of patients respond to Remicade. Side effects of Remicade include increased susceptibility to infectious disease, and it is important to develop predictors of Remicade responses. A20, a K63-deubiquitinase, inhibits TNF-directed NFkB activation in CD. Our studies show that A20 is phosphorylated in response to TNF activation. This phosphorylation site has been mapped, and we developed an to specifically detect this activated form of A20 (Hutti et al., MCB, in press). We hypothesize that phospho-A20 staining is decreased in Crohn's Disease patients treated with Remicade, and may serve as a prospective determinant of Remicade responses in patients with Crohn's Disease.

Design: This novel phospho-A20 antibody was used to stain 42 biopsy and resection specimens from patients either receiving or not receiving anti-TNF therapy. Longitudinal tissue samples were available from 10 patients and were examined in a longitudinal fashion (pre and post anti-TNF therapy) to examine changes in staining patterns as a result of treatment.

Results: 15/28 (54%) of patient tissue without anti-TNF therapy showed immunopositivity while 2/14 (14%) of patient tissue with anti-TNF therapy showed immunopositivity (p=0.0151; Fisher T-test). Longitudinally, 17% with positive immunostaining prior to therapy showed a poor response, while 50% with negative immunostaining prior to therapy showed a poor response. In 4/4 cases, Remicade treatment 3-weeks prior to biopsy altered the phospho-A20 staining from positive to negative. In all 4 cases, this staining pattern matched a clinical response to Remicade.

Conclusions: Phospho-A20 immunostaining can be utilized to distinguish anti-TNF therapy, and phospho-A20 immunostaining pattern can predict clinical responsiveness to anti-TNF therapy.

556 Lymphocytic Ileitis in Celiac Disease: A Marker of Severity, Distribution and Symptomatology

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Background: Celiac disease or gluten-sensitive enteropathy (GSE) is diagnosed by recognition of immune-mediated sensitivity to gluten, serological and HLA testing, and histological abnormalities in the proximal small bowel, which are graded by Marsh criteria (1-3c). More patients have atypical complaints rather than classical symptoms of diarrhea and weight loss. About 10-15% of patients with GSE are also said to have lymphocytic gastritis, and/or lymphocytic colitis. We investigated the relationship of lymphocytic ileitis and the severity, symptoms and distribution of GSE.

Design: 10 patients (4 males and 6 females, ages 30-66), underwent full colonoscopy for chronic diarrhea and random biopsies from their terminal ileum showed chronic lymphocytic ileitis with increased intraepithelial lymphocytes (IELs). Their concurrent random colonic biopsies, and concurrent or subsequent duodenal and gastric biopsies were reviewed for lymphocytic colitis, lymphocytic gastritis and lymphocytic duodenitis, and Marsh grade was performed on the duodenal biopsies. Their symptoms, celiac serology or HLA, and serum B12 levels were reviewed.

Results: Lymphocytic ileitis was diagnosed in 10 patients by finding diffusely increased IELs (>40%) in the terminal ileal biopsies along with moderate to severe increase in chronic inflammatory cells in the lamina propria and no shortening of villi. All 10 patients were clinically diagnosed as GSE based upon co-existent lymphocytic duodenitis (Marsh 3b or 3c in 5/10, Marsh 2 in 4/10 and Marsh 1 in 1/10) and positive serology (tTG-IgA and/or EMA-IgA) in 8/10 and positive HLA-DQ8 in the remaining 2. 8/10 (80%) patients had lymphocytic colitis, 5/10 (50%) patients had lymphocytic gastritis and 4/10 (40%) patients had both lymphocytic colitis and lymphocytic gastritis. 3/10 (30%) patients has low serum B12 levels (between 200-300 pg/mL). All patients had diarrhea and 6/10 (60%) patients also had weight loss.

Conclusions: Lymphocytic ileitis discovered during full colonoscopy and random sampling is a strong indicator of celiac disease and warrants serology and duodenal biopsies for confirmation of celiac. Patients with Lymphocytic ileitis also more often

have classical rather than atypical symptoms of celiac disease, higher Marsh grades in the duodenal biopsies and greater rate of co-existent lymphocytic gastritis and lymphocytic colitis than previously documented for typical GSE. Lymphocytic ileitis also carries a risk for B12 deficiency.

557 Dysfunction of Ileal Pouch-Anal Anastomosis in Ulcerative Colitis: Defining a Broader Clinico-Pathological Spectrum

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Background: A J-reservoir ileal pouch-anal anastomosis (IPAA) is a standard operative procedure after colectomy for ulcerative colitis (UC). About a third of patients may experience pouch dysfunction. The currently well-defined causes of pouch dysfunction include non-inflammatory irritable pouch syndrome (IPS), non-specific inflammation of pouch or pouchitis, persistent UC in the distal rectal cuff (cuffitis), and afferent limb ulcers (ALU) due to Crohn's or NSAIDs. In our study, we introduce additional entities.

Design: From 2000-2007, 44 patients (28 females and 16 males, ages 15-77) with colectomy for apparent UC had endoscopy and biopsies for pouch dysfunction (one or more symptoms such as diarrhea, urgency, bleeding and pain). The clinical features, endoscopic findings and images, precise biopsy sites in relationship to the pouch anatomy and routine HE stained slides were reviewed. In selected cases (7/44), the original clinical disease and the resected colon were reviewed.

Results: The endoscopies included descriptive evaluation of pouch, distal rectal cuff, blind loop and afferent limb (AL) to 50 cm, and biopsy specimens and sites varied depending upon the findings. The following clinico-pathological diagnoses were made: IPS 6/44 (14%), cuffitis 7/44 (16%), pouchitis 21/44 (48%) including "backwash" pouch ileitis in 7/21 cases of severe pouchitis which continuously involved 5-10 cm of distal AL, de novo eosinophilic enterocolitis in 2/44 (diffuse dense eosinophilia), "missed-Crohn's" in 2/44 or 5% (where colectomy was done less than 1 year ago and had more features of Crohn's than UC) and "neo-Crohn's" in 5/44 or 11% (where colectomy was done more than 4 years ago and had more features of UC than Crohn's). Diagnosis of Crohn's was based upon absence of NSAID use, patchy AL ileitis and ulceration proximal to 10 cm and/or clinically fistulae and/or perianal disease. Diagnosis of backwash pouch ileitis was based upon severe pouchitis continuously extending in AL for no more than 10 cm and lack of NSAID use or clinical features of Crohn's.

Conclusions: Histological pouch biopsy evaluation needs to be closely correlated with the clinical profile, endoscopic findings and sample sites from the pouch anatomy. In severe pouchitis, "backwash" pouch ileitis may occur analogous to backwash ileitis in pan-UC. Post UC "neo-Crohn's" can be differentiated from "missed-Crohn's" by longer post-colectomy interval and clinico-pathological confirmation of UC at the original colectomy. De novo diseases unrelated to IPAA are uncommon but may occur in the pouch.

558 Sp1, a New Biomarker for Aggressive Pancreatic Ductal Adenocarcinoma

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Background: Pancreatic carcinoma is one of the leading causes of cancer-related deaths in the United States. SP1 is a sequence-specific DNA binding protein that is important in the transcription of many genes that contain GC boxes in their promoter. In this study, we investigated whether Sp1 can serve as a biomarker to predict aggressive pancreatic adenocarcinoma.

Design: We studied 42 patients with primary pancreatic adenocarcinoma, ductal type, who underwent tumor resection with Whipple procedure. The tumor samples were obtained from the surgical pathology files of the University of Massachusetts Medical Center between 2000-2006. The over expression of Sp1 in the primary pancreatic adenocarcinomas was evaluated by immunohistochemical staining and 41 patients with clinical follow-up information were further evaluated for survival analysis.

Results: Sp1 over expression was significantly increased in a subset of primary pancreatic adenocarcinomas that were likely to develop metastasis. In fact, all tumors that expressed the Sp1 had lymph node metastasis, whereas none of the primary tumors without lymph node metastasis showed over expression of Sp1. Over expression of Sp1 was associated with higher tumor stage, grade and lymph node metastasis ($p<0.001$, $p=0.036$ and $p<0.0001$ respectively). Kaplan-Meier plots and log-rank tests in patients with pancreatic adenocarcinnoma showed that patients without over expression of Sp1 in their primary tumor had significant longer overall survival than patients with Sp1 over expression ($p=0.002$). The 5-year overall survival rate was 55% in patients without over expression of Sp1 versus 19% in patients with Sp1 over expression. The median survival was 65 months in patients without over expression of Sp1, whereas the median survival was only 13 months in patients with over expression of Sp1 in their primary tumors.

Conclusions: Sp1 is a new prognostic marker for the aggressive behavior of pancreatic adenocarcinoma. It can be used at initial diagnosis of pancreatic adenocarcinoma to identify a group of patients with a high chance to develop metastasis and died from the disease.

559 Coexisting C-KIT and PDGFRA Mutations in Gastrointestinal Stromal Tumors (GIST)

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Background: In spite of being relatively uncommon, gastrointestinal stromal tumors (GIST) have attracted considerable interest, as they are one of the first paradigms of targeted molecular therapies. Mutational studies have been found to be of prognostic value in these tumors. PDGFRA mutations are considered an alternative pathogenic pathway in GIST and their occurrence is said to be mutually exclusive with C-KIT mutations. However, reports analyzing both genes in the same tumors are scarce and contradictory. The aim of the present study has been to characterize the molecular changes in C-KIT and PDGFRA in a well defined subset of carefully cryopreserved GIST.

Design: A total of 15 GIST tumors are included. Tumor tissue was freshly retrieved and cryopreserved. C-KIT (exons 9 and 11) and PDGFRA (exons 12 and 18) mutations were assessed by PCR amplification and direct sequencing (ABIPRISM 377, Perkin-Elmer Applied Biosystems). The results were confirmed in two separate DNA extractions in each case. Mann-Whitney's non parametric test was used to assess the association of mutations with standard prognostic parameters (mitotic count/50 HPF, tumor size and location).

Results: C-KIT mutations were found in 10 cases, 9 of them in exon 11, and one in exon 9. Two other cases showed a mutation in exon 18 of PDGFRA. In 4 cases, an identical intronic mutation, not previously reported in this tumor, was found in exon 18 of PDGFRA. Interestingly, this intronic change coexisted with C-KIT exonic mutations in 3 cases. Finally, a polymorphism in exon 12 of PDGFRA was identified in all the 15 cases. Intestinal tumors often harbored intronic PDGFRA mutations. There was a statistically significant association between high mitotic counts and presence of C-KIT exon 11 mutations.

Conclusions: In this series, mutations in exon 11 of C-KIT are associated with desfavorable histologic features. Our results support the notion that exonic mutations are not found simultaneously in C-KIT and PDGFR. However, the coexistence of exonic and intronic changes in both genes in three of our cases is a novel finding that deserves further research and suggests more complex molecular mechanisms in these tumors. In conclusion, mutations of C-KIT and PDGFRA are not mutually exclusive in GIST.

560 Rectal Sparing and Skip Lesions in Ulcerative Colitis: A Comparative Study of Endoscopic and Histologic Findings in Patients Who Underwent Proctocolectomy

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Background: The prevalence rate of rectal sparing (RS) and skip lesions (SL) in treated ulcerative colitis (UC) ranges from 31-58%, but this is based on endoscopic or biopsy series. To date, no study has 1. evaluated these features in resection specimens, which, ultimately, is the pathologist's last opportunity to discriminate UC from Crohn's colitis (CC), and 2. evaluated the effects of immunomodulatory agents (anti-TNF α, cyclosporin A, 6MP etc.) on disease normalization. Therefore, these were the goals of this study.

Design: The study consisted of 56 UC patients (M/F=28/28, mean age=42 years) who underwent $\geq$ 1 preoperative endoscopies (n=168), with biopsies (n=512), prior to proctocolectomy. A wide variety of clinical (including type of treatment) and endoscopic parameters were correlated with the pathologic findings in consecutive biopsies, and in resection specimens. Histologic parameters of particular interest included relative RS (RRS) (inactive proctitis with proximal active colitis), absolute RS (ARS) (normal rectum), and SL (normal mucosa bordered on both sides by inactive or active colitis).

Results: The prevalence of RS and SL was 32.1% and 30.4% respectively, by endoscopy, 30.4% (ARS: 28%, RRS: 72%) and 25.0% (ARS: 45%, RRS: 55%), respectively, in biopsy series. In resection specimens, 12.5% showed SL, 5.4% showed RRS, but none (0%) showed ARS when all rectal tissue sections were considered. In terms of RS (either ARS or RRS), there was a strong significant correlation between the endoscopic and biopsy findings ($P=0.0001$), and between the finding of RS in biopsies and SL in the rectum of resection specimens ($P=0.006$). Clinically, there was a strong correlation between disease duration and RS by endoscopy ($P<0.01$) and biopsy ($P<0.01$), and with rectal SL in colectomies ($P=0.02$), but there was no correlation between the pathologic features and any type of medication, including immunomodulators or rectal enemas.

Conclusions: Apparent RS in either endoscopic or biopsy studies of UC patients does not indicate complete absence of rectal involvement, but it does correlate with the finding of rectal SL in the patient's colectomy specimen. Thus, the finding of rectal SL in colectomy specimens should not, by itself, mandate a change of diagnosis from UC to CC. Immunomodulatory agents have no effect on the prevalence rate of RS or SL in UC.

561 CpG Island Methylator Phenotype, Loss of Heterozygosity and BRAF/KRAS Mutations in Microsatellite Stable Colorectal Cancer: Correlation with Clinicopathologic Features and Survival

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Background: Microsatellite instability (MSI) and chromosomal instability (CI), the 2 main pathways in colorectal cancer (CRC), are not present in 20-37% of cases. We studied the clinicopathologic and molecular feaures of these tumors with an emphasis on CpG island methylator phenotype (CIMP) to investigate the role of aberrant methylation in this subgroup. We also examined the significance of CIMP pathway, CI and BRAF mutations in microsatellite-stable (MSS) cases.

Design: Chromosomal instability status was determined by loss of heterozygosity (LOH) analysis at 4 loci (5q,8p,17p,18q) using DNA extracted from 69 cases of formalin-fixed paraffin-embedded MSS colorectal cancer.CI was considered to be present if LOH was present in any of the four loci.Involvement of 2 or more loci by LOH was considered as high-level CI.MSS status was established by excluding MSI using Bethesda guidelines.CIMP had been previously determined using methylation specific PCR at 7 loci (MLH1, p16, MINT1, MINT31, MGMT, RASSF2, HIC1). Methylation at 3 or more loci was considered CIMP-high (CIMP-H). BRAF V600E and KRAS mutations were determined by PCR followed by sequencing. Tumor size, site, stage and survival were recorded for each case.

Results: Chromosomal instability manifested by LOH involving at least one locus was observed in 53 (77%) cases, while 16 (23%) MSS cases did not show LOH. The latter

group had a low incidence of CIMP-H (3/16,19%) and BRAF mutation (1/16,6%). Their 5-year survival was significantly better compared to MSS with LOH (80% vs. 54%,p=0.02). BRAF V600E mutations were seen in 10 (15%) MSS cases, and correlated significantly with high-level LOH (p=0.009) and poor 5-yr survival (0% vs. 70%, p<0.001). CIMP-H status correlated with BRAF V600E mutation (p=0.02), but not with clinicopathologic features, survival, LOH or KRAS mutations. In multivariate analysis, stage and BRAF V600E mutations were the only predictive factors.
Conclusions: CIMP-H as defined here does not play a significant role in MSS colorectal cancer lacking chromosomal instability determined by LOH analysis. These cases have better survival, likely related to absence of significant LOH. MSS cancers with BRAF V600E mutations have an adverse outcome, perhaps due to high-level LOH in these cases. CIMP-H as defined here correlates with BRAF V600E mutation, but does not show distinct clinical and pathologic features in MSS cases.

562 Distinct CpG Island Methylation Profile and BRAF Mutation Status in Serrated and Adenomatous Colorectal Polyps

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Background: CpG island methylator phenotype (CIMP) pathway in colorectal cancer is characterized by methylation of promoter regions of multiple putative tumor suppressor genes.This study systematically examines CIMP status and BRAF/KRAS mutations in different histologic types of serrated and adenomatous polyps.
Design: CIMP status was determined using DNA extracted from formalin-fixed paraffin-embedded tissue from 48 hyperplastic polyps (HP),32 sessile serrated adenomas,30 serrated adenomas,32 tubular adenomas (TA) and 32 tubulovillous/villous adenomas (TVA/VA) by methylation-specific PCR at 7 loci:*MLH1*, *p16*,*HIC1*,*RASSF2*,*MGMT*,*MINT1* and *MINT31*.Polyps with methylation at 3 or more loci were considered CIMP+. BRAF V600E and KRAS mutations were determined by PCR followed by sequencing. The influence of size and site of polyp on CIMP+ status was examined.Polyps >0.8cm were considered large.
Results: There was no significant difference in CIMP+ status in serrated and adenomatous polyps (39% vs 36%,p=0.4).*MGMT* methylation was more common in adenomatous polyps (60% vs 14%,p<0.001),while *MLH1*(15% vs 3%,p=0.01) and *HIC1*(38% vs 20%,p=0.01) were more often methylated in serrated polyps.Right-sided serrated polyps were more likely to be CIMP+(62% vs 22%,p<0.001),but side did not significantly affect CIMP+ in adenomatous polyps (43% vs 29%,p=0.2).CIMP+ was associated with large size in HP (p=0.01) and TVA/VA (p<0.001),but not in other polyps.BRAF V600E mutations were present in 80% of serrated polyps,but none of adenomatous polyps (p<0.001).KRAS mutations were seen in 10% of adenomatous polyps and 9% of serrated polyps.
Conclusions: There is no significant difference in CIMP-H status between serrated and adenomatous polyps.However,methylation affects different loci in serrated and adenomatous polyps:*MGMT* methylation is more common in adenomaous polyps,while *MLH1* and *HIC1* methylation is more often seen in serrated polyps.CIMP+ phenotype is more frequent on the right side in serrated polyps, but both right and left colon are similarly involved in adenomatous polyps.Methylation is associated with size in HP and TVA/VA.BRAF V600E mutations are characteristic of serrated polyps and are not observed in adenomatous polyps.

	HP	SSA	SA	TA	TVA/VA
Total %CIMP+	33	44	43	28	44
Right vs Left,%CIMP+	67/7	50/36	75/32	25/31	64/28
Large vs small,%CIMP+	60/21	46/42	46/42	36/24	50/0

563 Blood-Based Gene Array Biomarkers for Detection of Inflammatory Bowel Disease

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Background: Gene expression profiling is valuable to identify patterns of genes related to specific pathologic entities. However, the value of blood gene expression profiling has not been evaluated in patients with ulcerative colitis (UC) or Crohn's disease (CD). The aim of this prospective study was to evaluate genome-wide gene expression profiles in patients with inactive or active UC or CD and to compare the results with a control group of patients with diarrhea, but without inflammatory bowel disease (IBD).
Design: Genome-wide gene expression profiling was applied to blood from 20 patients with CD and 15 patients with UC in a blinded fashion. Mucosal biopsy samples were analyzed for the presence and degree of activity on a 3-point scale (CD= 7 inactive, 7 mildly active, 6 moderate/severe activity; UC=7 inactive, 1 mildly active and 7 moderate/severe activity). Eight control patients with diarrhea but without colonic pathology were evaluated as controls. Genome-wide expression profiles generated with Affymetrix GeneChip U133Plus2.0 were analyzed and correlated between IBD and non-IBD patients, and between CD and UC patients with similar degrees of activity.
Results: Four-hundred and eighty-five unique genes were significantly (ANOVA: P<0.01) differentially expressed in different CD severity groups and non-IBD controls of which approximately half were differentially expressed in active CD and half correlated with disease activity. These included 9 genes involved in the IL-6 signaling pathway and 8 genes involved in VEGF signaling pathways. In UC, 60 unique genes were significantly differentially expressed in UC blood compared to controls, with the largest differences present in active UC. Of note, 6 genes involved in natural killer cell signaling were down-regulated in UC. Finally, 172 genes were significantly differentially expressed in active CD vs. active UC and 48 genes in inactive CD vs. inactive UC. More specifically, genes involved in peroxisome proliferator-activated receptor α activation pathways (MAPK1, MAP2K7, PLCB1) were up-regulated in active CD compared to UC, whereas two different genes in this pathway (ACOX1, MAP2K3) were down-regulated in inactive CD compared to inactive UC.
Conclusions: In this initial discovery study, gene array analysis shows promise as both a diagnostic tool and as a method for uncovering genes involved in the pathogenesis of IBD. Further studies utilizing larger groups of patients should be performed to confirm our findings.

564 Comparative Analysis of Three Different Panels for CpG Island Methylator Phenotype in Colorectal Cancer

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Background: The CpG island methylator phenotype (CIMP) represents a distinct subset of colorectal cancers (CRCs). The CIMP CRCs are hypermethylated in multiple CpG island loci and exhibit characteristic clinicopathologic profiles. However, a selection of markers to determine CIMP has been controversial. Previous studies have suggested valuable markers for CIMP including MINT1, MINT2, MINT31, p16, MLH1, CRABP1, CACNA1G, NEUROG1, SOCS1, IGF2 and RUNX3. The aims of our study is to validate three different CIMP marker panels through correlation with clinical features using quantitative methylation analysis.
Design: A total of 112 CRC specimens were included in this study. To assess methylation of CIMP markers, MethyLight assay was performed. CIMP positivity was defined by three different CIMP marker panels. Statistical analysis was carried out for the correlation of CIMP with clinicopathologic features-age, gender, gross tumor type, histologic tumor differentiation and survival.
Results: We compared three representative CIMP marker groups-the classic panel (group 1; including MINT1, MINT2, MINT31, p16 and MLH1), recently proposed panels by Ogino et al. (group 2; p16, MLH1, CRABP1, CACNA1G and NEUROG1) and Laird et al. (group 3; CACNA1G, NEUROG1, SOCS1, IGF2 and RUNX3). Among these marker groups, the group 2 markers showed the most prominent relationship to clinicopathologic features known to be typical CIMP characteristics. The group 2 markers were highly correlated with old age, female patients, ulceroinfiltrative tumor type and poor differentiation than other three marker groups. Interestingly, the group 2 markers were closely associated with patient survival. CIMP CRCs determined by group 2 markers exhibited poorer survival than non-CIMP CRCs with statistical significance (p=0.0198). We also analyzed each CIMP markers and additional CpG island loci which had seemed to be related to survival in our previous study and found that methylation of RUNX3, Bcl2 and BDNF were significantly correlated with worse survival.
Conclusions: The results indicate that Ognino's panel (p16, MLH1, CRABP1, CACNA1G and NEUROG1) well characterized CIMP in CRC. The CIMP CRCs defined by Ogino's marker panel was closely associated with old age, female, proximal location, poor differentiation, and poor prognosis.

565 Microsatellite Instability-High Colorectal Cancers in Korean Patients: Its Detection and Clinicopathologic Features

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Background: Microsatellites instability (MSI) is an indicator of deficient mismatch repair (MMR) that is associated with germline mutation of MMR genes or epigenetic silencing of MLH1. A large fraction of MSI-high (MSI-H) tumors are known to be sporadic cases that have better prognosis than microsatellite stable (MSS) tumors. We analyzed MSI test of 216 Korean patients with colorectal cancer(s) to know the incidence and clinicopatholgic features of MSI-H colorectal cancers.
Design: MSI test was performed by using a panel of microsatellites (BAT25, BAT26, D5S346, D2S123, and D17S250) and the results were classified into MSS, MSI-low (MSI-L), and MSI-H according to the recommendations by National Cancer Institute. The expressions of MLH1 and MSH2 protein were evaluated immunohistochemically. In patients with two or more synchronous or metachronous colorectal cancers, results for the most advanced lesions were included for statistical analysis.
Results: Among 216 colorectal cancer patients, 25 (11.6%) and 4 (1.9%) patients had MSI-H and MSI-L tumors, respectively. All MSI-H tumors showed mutations in microsatellites markers with mononucleotide repeats (100% in BAT26 and 92% in BAT25). None of MSI-L tumors showed mutation in BAT26 and two (50%) MSI-L tumors had mutations in BAT25. Among MSI-H tumors, one (4%) patient met Amsterdam criteria ll and 5 (20%) patients had two or three colorectal cancers. MSI-H tumors were associated with younger patients' age (p=0.003), multiplicity (p=0.001), proximal colon location (p<0.001), poor histologic differentiation (p<0.001) and mucinous/signet ring cell component (p=0.001). However, there was no difference in depth of tumor invasion, regional lymph node status and distant metastasis between MSI-H and MSS tumors. Loss of MLH1 and/or MSH2 protein expression was found in 92% of MSI-H, 0% of MSI-L and 1.8% of MSS tumors (p<0.001).
Conclusions: We detected MSI-H tumors in 11.6% of Korean colorectal cancers, and its proportion is just similar to those of Western countries. BAT26 was the most sensitive microsatellite marker for detecting MSI-H tumors. Most of MSI-H tumors seemed to be clinically sporadic in Korea although mutation analysis for MMR genes is needed. MSI-H tumors showed several differences in clinical presentation compared with MSS tumors, Loss of MLH1 and/or MSH2 protein expression can be a powerful predictor of MSI-H status in colorectal cancers.

566 *Helicobacter pylori* Infection in the Stomach: Is This an Invasive Organism?

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Background: *Helicobacter pylori* is associated with chronic active gastritis and is also considered a major factor in the development of gastric adenocarcinoma. The organism is usually considered a non-invasive colonizer of the gastric mucosa, but it

has recently been documented that *H. pylori* has the potential for tissue invasion. We sought to determine the frequency of invasive *H. pylori* in various pathologic conditions associated with development of gastric carcinoma.
Design: We retrieved gastric tissues from 90 cases, including unremarkable gastric mucosa (n= 10), *H. pylori* associated chronic active gastritis (n = 25), multifocal atrophic gastritis (n = 14), and gastric carcinoma (n = 41). All sections were stained for *H. pylori* organisms using standard immunohistochemical techniques. The presence and location of *H. pylori* organisms were evaluated by two reviewers. Clinicopathologic parameters were recorded, and univariate analysis was performed to determine association of prognosis with invasion of *H. pylori* into the lamina propria.
Results: Patients whose gastric tissues were included had a mean age of 52 years (range: 20 to 89 years), including gastric carcinoma patients with a mean age of 64 years (range: 21 to 88 years). Invasive *H. pylori* in the lamina propria was identified in 26 of 90 cases (29%). Invasion of *H. pylori* was noted significantly more frequently in *H. pylori* associated chronic active gastritis (13/25, 52%) in comparison to both multifocal atrophic gastritis (2/14, 14%) and gastric carcinoma (10/41, 24%) ($p<0.05$). No *H. pylori* organisms were identified in any of the sections of unremarkable gastric mucosa. Invasive *H. pylori* was almost always associated with surface *H. pylori* (25/26, 96%). Only coccoid morphology was noted for organisms demonstrating invasion into the lamina propria. *H. pylori* invasion did not affect prognosis in gastric carcinoma ($p>0.05$).
Conclusions: Invasion of *H. pylori* into the gastric lamina propria is not an uncommon finding in chronic active gastritis and gastric carcinoma. This invasion may contribute to the inflammatory response incited by the organism and to the pathogenesis of gastric carcinoma, and this phenomenon warrants further investigation. The presence of invasive *H. pylori* was not found to be a significant prognostic factor in gastric adenocarcinoma.

567 Surface SMAD4 Protein Expression in Hyperplastic Polyps of the Stomach

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Background: Mutations in the SMAD4 gene (DPC4) on chromosome 18q21 have been associated with pancreatic and colorectal cancer. It has been shown that loss of heterozygosity in SMAD4$^{+/-}$ mice resulted in up-regulation of gastric hyperplastic epithelial proliferation and invasive carcinoma. A recent study has demonstrated that knocking out of the SMAD4 gene is associated with hyperplastic polyps in the stomachs of mice (Cancer Res. 2007; 67:8121-30). The aim of this study was to determine the association of SMAD4 protein expression in gastric hyperplastic polyps in human tissues.
Design: We retrieved 20 gastric hyperplastic polyps and 30 control antral gastric tissues including unremarkable gastric mucosa (n= 10) and chemical gastritis demonstrating foveolar hyperplasia (n= 20). All tissues were reviewed for associated chronic and active inflammation as well as H.pylori infection. Serial sections were immunohistochemically stained for SMAD4 protein. The tissue was analyzed for the presence of SMAD4 in superficial and deep epithelial tissue. A nuclear and cytoplasmic staining intensity score was given for each hyperplastic polyp and each control case. Staining was considered positive when greater than 10% of the cells stained for SMAD4 protein. A Chi - squared test was performed to determine significant differences in expression pattern.
Results: The mean age of patients with hyperplastic polyps was 58 years (range: 39-86 years). The mean age of patients in the control group was 47 years (range: 22 - 84 years). H.pylori organisms were not indeitified in the hyperplastic polyps or in the control group. Lack of nuclear SMAD4 protein expression was significantly ($p = 0.025$) more frequently noted in superficial foveolar mucosa of hyperplastic polyps (19/20; 95%) in comparison to the control group (5/30; 17%). No significant difference was noted in the nuclear or cytoplasmic expression of SMAD4 in deep epithelial cells of the hyperplastic polyps and the control group. In 11/20 cases both nuclear and cytoplasmic expression was noted in deep epithelial cells of the hyperplastic polyps.
Conclusions: Loss of SMAD4 expression is associated with gastric hyperplastic polyps in humans. The loss of this tumor suppressor protein may be one of the key steps in the progression from normal gastric epithelium to hyperplasia, and eventually invasive carcinoma in humans.

568 Molecular Features of Serrated Adenomas from Korea

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Background: Anecdotal observations suggest that colonic serrated adenomas (SA) are more prevalent in the Far East compared to North America, which may be due to differences in diet or genetic background. However, the molecular properties of SA's in the Far East, such as Korea, are unknown. The aim of this study was to evaluate the molecular features of SA's with various degrees of neoplastic progression, and to compare the features with conventional adenomas (CA) from the same geographic region.
Design: One-hundred and fifteen SA's from both the right and left colon, were categorized pathologically as either low-grade (N=58), high-grade ("Advanced") (N=28), SA with conventional adenoma (CA) cytologic features focally (SA-CA) (N=20), SA with adenocarcinoma (SA-Adca) (N=9), and evaluated for the presence of K-RAS and BRAF mutations, and for methylation of MGMT, MLH1 and APC. In a subset of cases, microdissected regions of adjacent hyperplastic polyp (HP) appearing (non-dysplastic) mucosa was also sampled. The data was compared between the different histologic sub-types, and with 26 CA's.
Results: Mutations of K-RAS or BRAF, and methylation of MGMT, MLH 1 and APC, were present in 17%, 72%, 48%, 7% and 33% of low-grade SA's, respectively. High-grade (advanced) SA's showed a higher rate of K-RAS, and a lower rate of BRAF, mutations, but were otherwise similar. Both SA-CA and with SA-Adca also showed a higher rate of K-RAS mutations (35% and 44% respectively, $P<0.05$) and a lower rate of BRAF mutations (33% and 33% respectively, $P<0.05$) compared to SA's. No differences were observed between SA-CA and SA-Adca. In comparison to SA's, CA's also showed a significantly higher rate of K-RAS mutations (33%) and a lower rate of BRAF mutations (0%), but also a higher rate of APC methylation (67%). Regardless of histologic sub-types, K-RAS mutations were more prevalent in the left colon ($P<0.001$), whereas BRAF mutations and MLH1 methylation were more common in the right and transverse colon ($P<0.005$). Neither methylation of APC or MGMT showed a site prediction. Forty-three percent of SA's showed adjacent HP-appearing epithelium and, in general, mutations present in the dysplastic portion of the polyps were also present in the HP areas.
Conclusions: Unlike SA's from North American individuals, SA's in Korean patients show a higher prevalence rate of BRAF mutations and MGMT methylation, and APC methylation. These mutations occur early in the course of serrated carcinogenesis. K-RAS mutation may herald the onset of an aggressive phenotype in SA's from Korea.

569 The Senescence Related Gene RSK4 Is Downregulated in Colon and Renal Cell Carcinomas and Induces Senescence When Overexpressed

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Background: The study of senescence markers and their biochemical pathways is still at the beginning. In order to acquire full malignant capabilities cells should avoid replicative and oncogenic induced senescence. In a large RNAi screen, RSK4 gene was found to be related to p53 dependent arrest. RSK4 belongs to the RSK family (p90 rsk), but inhibits MAPK activity.
Design: The aim of this study was to study the level of expression of RSK4 in a series of human carcinomas and to analyze its role in senescence. 30 colon carcinomas, 8 colon adenomas, 20 renal cell carcinomas and 20 lung carcinomas were analyzed by mRNA and protein expression. mRNA was extracted from both normal and tumor tissues of each patient. Real time PCR using TaqMan probes for RSK4 was carried out. Western-blot of RSK4, p16, p21 and p53 was performed in tumor samples and cell lines. Cell lines studied included normal fibroblasts and colon carcinoma HCT116 (null p53) and HCT116 (wtp53).
Results: RSK4 gene was donwregulated, both mRNA and protein, in 27/30 colon carcinomas (p 0,001), in 15/20 renal cell carcinomas (p 0,05) and in 5/8 colon adenomas It was donwregulated in only 50% of the lung carcinomas. In vitro, overexpression of RSK4 induced cell arrest and senescence features in colon carcinoma cell lines. Moreover, in stress induced senescence there was an increase (up 12 folds) in mRNA RSK levels.
Conclusions: In most colon and renal cell carcinomas, RSK4 is downregulated. Moreover, overexpression of RSK4 induces senescence in colon carcinomas cell lines. These results indicate that RSK4 may be a crucial factor in pathways driving cells to senescence and support that RSK4 may be a tumor suppresor gene. Biochemical upregulating of RSK4 may be a novel therapeutical approach.

570 Histopathologic Features and Clinical Correlates of Small Intestinal Bacterial Overgrowth

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Background: Small intestinal bacterial overgrowth (SIBO) - defined as duodenal fluid culture growing $>10^5$ colony forming units (CFUs) of bacteria - is a common cause of chronic diarrhea. Morphologic changes in small bowel biopsies are not always seen in the setting of SIBO and morphologic changes that are associated with this condition have not been studied in detail.
Design: We conducted a retrospective search of our Microbiology database for patients with duodenal aspirate cultures during 2006. Patients were excluded if small intestinal biopsies were unavailable or if there was a clinical diagnosis of celiac sprue. The final study population comprised 69 patients with positive cultures ($>10^5$ CFUs) and 56 controls with negative cultures ($<10^5$ CFUs). Each small intestinal biopsy was assessed for the following hitologic features: 1) villous:crypt (v:c) ratio, 2) intraepithelial lymphocytosis (>20 per 100 epithelial cells), 3) crypt apoptoses (>5 per 10 crypts), 4) basal plasmacytosis, 5) neutrophilic cryptitis and villitis, 6) peptic duodenitis 7) erosions and ulcers, and 8) increased eosinophils. Clinical records were reviewed for presenting symptoms and diagnosis.
Results: The only histologic feature that differed between SIBO and controls was a v:c ratio of <3:1, seen in 17 (25%) patients with SIBO and 4 (7%) controls ($p = 0.015$). There were no significant differences in intraepithelial lymphocytosis (20% of SIBO vs. 14% controls, p=0.48), cypt apoptoses (4% vs. 5%, p=1.0), basal plasmacytosis (9% vs. 4%, p=0.3), neurophilic cryptitis/villitis (6% vs. 5%, p=1.0), peptic duodenitis (4% vs. 9%, p=0.7), or increased eosinophils (16% vs.14%, p=1.0). Overall, we judged the small intestinal biopsies to be within normal limits in 46 (67%) of patients with SIBO and 41 (73%) controls (p=0.44). Clinically, diarrhea and abdominal pain were the most common symptoms, both in patients with SIBO (52% and 25%, respectively) and controls (55% and 23%, respectively).
Conclusions: Most (67%) small intestinal biopsies in the setting of SIBO are histologically within normal limits. A decreased v:c ratio is the most common finding (25% of all SIBO cases) and the one that best distinguishes SIBO from nonceliac cases with negative cultures (p=0.015). Overall, both the histologic features and the clinical symptoms in patients with SIBO are nonspecific. Therefore, SIBO needs to be considered as a potential etiology for gastrointestinal symptoms even when duodenal biopsies are normal.

571 Immunohistochemical Phenotypes of Pancreatic Endocrine Neoplasms (PENs) and Other Pancreatic Tumors That PENs Can Histologically Mimic

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Background: Differentiating pancreatic endocrine neoplasms (PENs) from other pancreatic tumors on morphology alone can be difficult, particularly on needle biopsy. The histological features of PENS can mimic those of other pancreatic tumors. Furthermore, limited studies suggest that the immunohistochemical profile of PENs and histologically similar tumors can sometimes overlap, potentially leading to misdiagnosis. The purpose of this study was to compare immunohistochemical patterns of PENs with those of solid pseudopapillary tumors, acinar cell carcinoma, and pancreatoblastoma.

Design: Tissue cores were collected from routinely processed paraffin-embedded tissue of 59 PENs, 10 solid pseudopapillary tumors (SPTs), 2 acinar cell carcinomas (ACCs) and 1 pancreatoblastoma (PB). Five 2 mm tissue cores were taken from random areas of the tumor, arranged in tissue microarrays (TMAs), and immunohistochemically stained for pan-CK, CK7, CK19, CK20, CDX-2, CA19.9, synaptophysin, chromogranin, carcinoembryonic antigen (CEA), CD10, and vimentin.

Results: All PENs were positive for either chromogranin (90%, 53/59) or synaptophysin (98%, 58/59). The majority of the PENs stained for pan-CK (75%, 44/59) and vimentin (61%, 36/59), but only a minority stained for CK7 (15%, 9/59), CK20 (10%, 6/59), CK19 (31%, 18/59), CDX-2 (7%, 4/59), CEA (29%, 17/59), and CD10 (20%, 12/59). Nearly half of the PENs stained for CA19.9 (49%, 29/59). All SPTs were positive for CD10 and vimentin. 40% (4/10) of the SPTs were positive for synaptophysin and one SPT stained for pan-CK. The SPTs were negative for chromogranin, pan-CK, CK7, CK19, CK20, CDX-2, CA19.9, and CEA. Both ACCs were positive for pan-CK, CK7, and CA19.9, and one ACC was positive for CD10. The ACCs were negative for all other markers. The PB was positive for all markers except CK20 and vimentin.

Conclusions: Our study demonstrates that distinguishing PENs from SPTs, ACCs and PBs can be further complicated by shared immunoprofile expression. PENs and SPTs showed overlap in staining for CD10, vimentin, and synaptophysin. The immunoprofiles of ACCs and PENs also showed similarities with both staining for pan-CK, CK7, CA19.9, although chromogranin and synaptophysin were negative in both ACCs. Lastly, the PB exhibited significant immunohistochemical overlap with PENs, staining with all immunomarkers except CK20 and vimentin. Thus, performing a thorough immunohistochemical work-up is essential when differentiating PENs and the tumors they can mimic.

572 Expression of Paxillin and Galectin-3 Closely Correlated with Patients' Survival in Colorectal Adenocarcinoma

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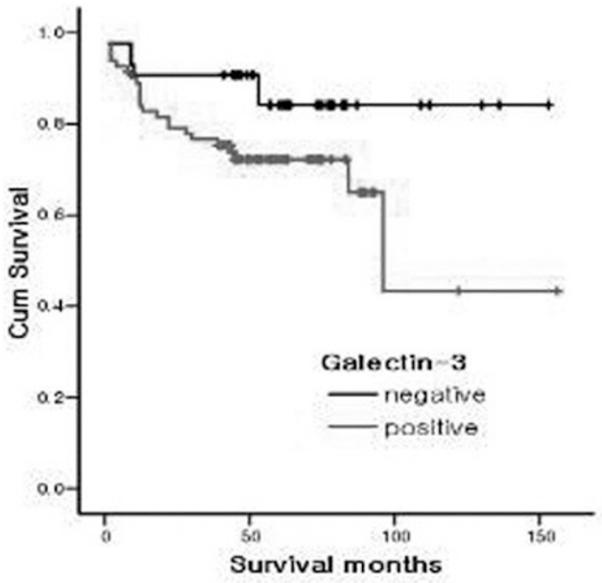
Background: The focal adhesion complex including paxillin is an important link between the actin cytoskeleton and the plasma membrane and proteins that assemble at sites of attachment of the cell to the extracellular matrix. Galectin-3 is a β-galactoside binding protein and expressed at elevated levels in a variety of neoplastic cells. The aim of this study was to investigate, by clinicopathologic and survival analysis, the correlation between the tumor progression and prognosis and the expression of paxillin and galectin-3 in colorectal cancer.

Design: Expression of paxillin and galectin-3 was evaluated using immunohistochemistry in 125 patients with colorectal adenocarcinoma. The results were correlated with both clinicopathological parameters (sex, location, histologic grade, tumor size, tumor depth, lymph node metastasis, distant metastasis and stage) and the overall survival.

Results: The paxillin expression was positive in 67 cases (53.6%) and significantly correlated with a smaller tumor size (p=0.001), more superficial tumor depth (p=0.007) and lower stage (p=0.03). The galectin-3 expression was positive in 82 cases (65.6%) and significantly correlated with deeper tumor invasion (p=0.000), lymph node metastasis (p=0.000), higher tumor stage (p=0.000). In a univariate survival analysis, lymph node metastasis (p=0.001), distant metastasis (p=0.000), stage (p=0.000) and galectin-3 expression (p=0.04) were independent predictors of patient outcome.

Conclusions: These findings indicate that the decreased paxillin expression and increased galectin-3 expression may play an important role in the tumor invasion and progression. Paxillin expression may be associated with more favorable prognosis. Galectin-3 expression may be associated with unfavorable prognosis. They can be used as valuable biomarkers to predict the prognosis of patients with colorectal adenocarcinoma.



573 Expression of CDX2 and Ets-1 Closely Correlated with Patients' Survival in Gastric Adenocarcinoma

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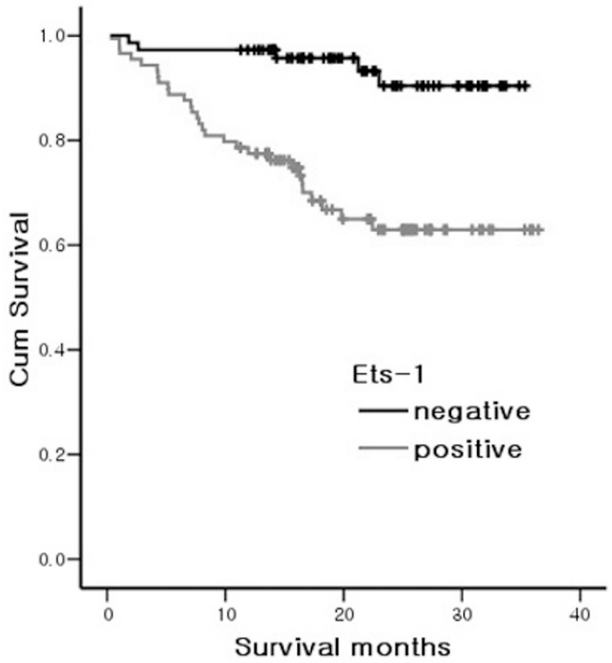
Background: CDX2, a transcription factor expressed in the intestine, is an important regulator of intestinal differentiation, which was previously identified in gastric carcinomas and intestinal metaplasia. Ets-1 protein is a transcription factor that has been implicated in both tumor invasion and angiogenesis. The aim of this study was to evaluate the role of CDX2 and Ets-1 as a predictor for cancer progression and prognosis in gastric adenocarcinoma.

Design: Expression of CDX2 and Ets-1 was evaluated using immunohistochemistry in 163 cases of primary gastric adenocarcinoma. The immuostaining results were correlated with both clinicopathological parameters (age, sex, Lauren classification type, histologic grade, tumor depth, lymph node metastasis, distant metastasis and stage) and the overall survival.

Results: CDX2 expression was positive in 93 cases (57.1%) and significantly correlated with intestinal type (p=0.02), superficial tumor depth (p=0.01), no node metastasis (p=0.02), lower stage (p=0.01) and longer survival rate (p=0.05). Ets-1 was not expressed in benign gastric epithelium. Ets-1 expression was positive in 89 cases (54.6%) and significantly correlated with deeper tumor invasion (p<0.0001), lymph node metastasis (p<0.0001), higher stage (p<0.0001) and shorter survival rate (p<0.0001). In a univariate survival analysis, tumor depth, lymph node metastasis, tumor stage, CDX2 and Ets-1 expression were independent predictors of patient outcome.

Conclusions: These findings indicate that CDX2 may be involved in intestinal differentiation. The loss of CDX2 expression and Ets-1 expression may play a role in the tumor invasion and lymph node metastasis. The CDX2 expression may be associated with more favorable prognosis and Ets-1 expression may be associated with unfavorable prognosis. Therefore, CDX2 and Ets-1 can be used as valuable markers to predict the outcome for patients with gastric adenocarcinoma.



574 Heat Shock Protein 90 Overexpression Independently Predicts Inferior Disease-Free Survival in Gastrointestinal Stromal Tumors (GISTs)

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Background: Most GISTs display oncogenic mutations of the receptor tyrosine kinases (RTKs), ie, *KIT* or *PDGFRA* gene. Heat shock protein 90 (HSP90) is a chaperone mediating the correct folding and stabilization of client proteins involved in tumorigenesis, including KIT proteins. HSP 90 inhibition by its antagonist, 17-AAG, can attenuate cell proliferation and survival in KIT-positive GIST cell lines. However, no study has attempted to evaluate the expression and significance of HSP90 in GIST specimens.

Design: Immunohistochemistry was performed for HSP90 in tissue microarrays of 306 primary localized GISTs, 165 of which were screened by DHPLC with confirmatory sequencing for *KIT* exons 9, 11,13, 17 and *PDGFRA* exons 12, 18. Follow-up was obtained in 281 cases with a median of 37.8 months. HSP90 expression was correlated with NIH criteria, Ki-67 labeling index (LI), RTK mutation types, and disease-free survival (DFS).

Results: HSP90 overexpression was seen in 167/306 cases and significantly associated with the intestinal site, larger size, and higher mitotic count, Ki-67 LI, and NIH risk category ($p< or = 0.001$ for all). Compared to the *KIT* exon 11 mutants having point mutation with or without 3'-duplication, HSP90 was preferentially overexpressed in GISTs showing wild genotype, 5'-deletion in *KIT* exon11, or other types of mutated RTK ($p=0.012$). HSP90 overexpression strongly correlated with decreased DFS ($p<0.0001$) by log rank test and independently predicted increased risk of adverse outcome ($p=0.023$, risk ratio [RR]=1.80) in multivariate analysis, together with high risk category ($p<0.001$, RR=3.22), Ki-67 LI>5% ($p=0.001$, RR=2.34), and intestinal location ($p=0.015$, RR=1.75).

Conclusions: HSP90 is overexpressed in 55% of GISTs and represents an independent prognosticator that correlates with NIH risk category, cell proliferation, and RTK mutation types. The findings reinforce the role of HSP90 in disease progression and provide an alternative drug target for high-risk, imatinib-resistant GISTs.

575 Aeromonas: An Emerging Food-Borne Cause of Infectious Colitis

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Background: Aeromonas species, initially thought to be nonpathogenic bacteria, are increasingly clinically recognized as a cause of food-borne enterocolitis. The typical presentation is diarrhea, sometimes accompanied by nausea, vomiting, and cramping pain, and symptoms may persist for days to months. The colonoscopic findings can mimic chronic idiopathic inflammatory bowel disease. We report the first series of Aeromonas colitis cases confirmed by both microbiological and molecular methods.

Design: Four culture-proven cases of Aeromonas infection with corresponding colon biopsies were retrieved, from 2001-2004. PCR and fragment analysis were performed following DNA extraction from the paraffin block; primers detected a 177-bp fragment of the flagellin gene specific to Aeromonas hydrophila. Biopsies and available medical records were reviewed.

Results: All four culture proven cases were confirmed by PCR/fragment analysis. Pertinent features of the cases are given in the table below. Patient 1 was thought to have refractory C. difficile colitis. Patient 2 had a history of quiescent ulcerative colitis with sudden flare; biopsy showed segmental ulceration superimposed on chronic colitis. Patients 3 and 4 had no previous GI history.

Conclusions: Rare case reports of Aeromonas infection are present in the clinical literature (including cases associated with ulcerative colitis). However, we present the first series of cases confirmed with both microbiological and molecular methods, and with corresponding biopsies. Although a small series, these cases suggest that Aeromonas may cause focal changes of chronicity as well as colonoscopic findings that can mimic chronic idiopathic inflammatory bowel disease. In addition, Aeromonas could be implicated in flares of ulcerative colitis. Pathologists should be aware of Aeromonas as a potential cause of infectious colitis, for it is easily cultured and easily treated with antibiotics.

Features of Patients with Aeromonas Colitis

Case #	Age/ gender	Symptoms	Colonoscopy	Pathology	Outcome
1	54F	Months of persistent diarrhea	Sigmoid erythema	Regenerative changes	Resolved w/ abx
2	17M	Diarrhea, 2 days	Diffuse friability	Ulcer overlying CUC	Unknown tx
3	55F	Diarrhea	Patchy erythema throughout	Focal cryptitis	Resolved w/ abx
4	62M	Diarrhea, one week	Right side colitis with ulcers, ?Crohn's	Focal cryptitis with architectural distortion	Resolved w/ abx

576 Tissue Polarity Protein Lgl2 Is Specific for Gastric Epithelium and Is Lost in Gastric Dysplasia and Carcinoma

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Background: Gastric dysplasia (GD) is a precursor lesion of gastric carcinoma (GC). Reliable histologic diagnosis of GD is hampered by interobserver variability and by resemblance to regenerative change. An important marker of dysplasia is loss of cell polarity. However, abnormal cell polarity may be difficult to ascertain in GD, especially in the setting of inflammation or injury. Utilization of a biomarker of cell polarity could be useful in the diagnosis of GD. One gene involved in maintenance of epithelial cell polarity and proliferation is Lethal giant larvae (Lgl). Lgl exerts its function by localizing to the submembranous basolateral cell domain. Two homologs, Lgl1 and Lgl2, are present in mammals and Lgl2 mRNA was shown to be highly expressed in the stomach. We hypothesized that Lgl2 protein expression and/or localization is disrupted in gastric dysplasia-cancer progression. The goals of our study were to evaluate the specificity and patterns of Lgl2 expression in normal gastrointestinal epithelia, in regenerative change of chemical gastropathy (ChG), in GD and GC.

Design: Routinely processed biopsy and resection specimens including 72 normal mucosa (8 esophagus, 41 stomach [14 antrum, 14 body/fundus and 13 cardia], 11 duodenum, 12 colon); 10 ChG, 15 GD (11 gastric adenomas, 4 foci of flat GD adjacent to intestinal-type GC) and 16 GC (8 intestinal-type and 8 diffuse-type) were immunostained using a monoclonal anti-Lgl2 antibody. Lgl2 expression was scored as basolateral or diffuse and as strong, moderate or weak.

Results: All normal gastric epithelia regardless of site and all 10 ChG showed strong/ moderate basolateral Lgl2 staining. Lgl2 staining was absent in 81% or mislocalized in 19% cases of GD and GC, as shown in the Table. Areas of intestinal metaplasia, present in 16 GD and GC, and all normal duodenal, colonic and esophageal mucosae, were negative for Lgl2.

Lgl2 staining patterns in ChG, GD and GC

	ChG (n=10)	GD		GC	
		Adenoma (n=11)	Flat GD (n=4)	Diffuse (n=8)	Intestinal (n=8)
Basolateral strong/moderate	10	0	0	0	0
Diffuse weak	0	0	3	0	3[1]
Negative	0	11	1	8	5[2]

[1]All GCs were well-differentiated. [2]Three GCs were moderately differentiated and 2 poorly differentiated.

Conclusions: Our data suggest that Lgl2 cell polarity protein is expressed specifically in normal gastric mucosa, is preserved in ChG and is lost or mislocalized in GD and GC. We propose that Lgl2 is a marker that could help in the diagnosis of GD and in distinguishing GD from regenerative change.

577 Expression of Phosphatase Regenerating Liver -1 and -3 Proteins in Esophageal Squamous Cell Carcinoma

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Background: Phosphatase of regenerating liver (PRL) is a new subclass of protein tyrosine phosphatase family and consists of 3 members so far, PRL-1, PRL-2, and PRL-3. Aberrant expression of PRLs has been identified in human colorectal, breast, hepatic, and ovarian cancers, but not reported in human esophageal squamous cell carcinoma (ESCC).

Design: Expression of PRL-1 and PRL-3 proteins was examined with immunohistochemistry in 60 cases of ESCC with matched lymph node metastasis (N=40), 6 esophageal adenocarcinoma, and 4 cases of esophageal dysplasia. The association of expression of PRL-1 and PRL-3 proteins with clinicopathological parameters was analyzed.

Results: PRL-1 and -3 proteins were expressed in the basal cell layers of dysplastic but not in the normal esophageal epithelium. The frequencies of PRL-1 and PRL-3 protein expressions were significantly higher in ESCC with lymph node metastasis than those without lymph node metastasis ($p<0.05$), also significantly higher in metastatic ESCC in lymph node than that in primary ESCC ($p<0.05$). Expression of PRL-1 and PRL-3

proteins correlated with the differentiation of ESCC, more often seen in well than moderately and/or poorly differentiated tumors. PRL-3 protein tended to associated with the depth of tumor invasion, but without significant difference. The frequencies of PRL-1 and PRL-3 protein expression were not associated with the patients' age and sex or tumor size.

Conclusions: 1). Expression of PRL-1 and PRL-3 protein may aid in the diagnosis of esophageal dysplasia. 2). PRL-1 and PRL-3 may be involved in the metastasis of ESCC. 3). PRL-1 and PRL-3 may serve as the biomarker for prediction of tumor metastasis of ESCC. 4). PRL-1 and PRL-3 have the potential to serve as therapeutic targets in ESCC.

578 Lymphangiomas in the Gastrointestinal Tract: A Comparison between Adults and Children

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Background: Lymphangiomas are rare tumors that arise from lymphatic channels and are usually found in the head and neck. Gastrointestinal (GI) involvement, although uncommon, is recognized with increasing frequency as more patients undergo GI endoscopic evaluations.

Design: We conducted a retrospective review at the University of Washington and Children's Hospital and Regional Medical Center to identify cases with the diagnoses of lymphangioma or lymphangiectasia in the GI tract, between 1982 and 2007. The clinical, endoscopic, and histologic findings were characterized.

Results: 32 specimens were identified, including 9 (4 resection; 5 biopsies) adult lymphangiomas (ALO), 12 (all resections) pediatric lymphangiomas (PLO), and 11 (all biopsies) adult lymphangiectasias (ALA). Males and females were equal (PLO 6:5; ALO 4:5) in both groups, whereas females predominated (2:9) in the ALA group. ALO and PLO averaged 59 and 4.5 years, respectively, and ALA averaged 60 years. The average ALO measured 1.4 cm and presented in the small bowel (SB, n=6), stomach (n=2), and sigmoid (n=1) as polypoid masses on endoscopy. The average PLO measured 5 cm and presented in the mesentery (n=5), abdominal wall (n=3), SB (n=3), or retroperitoneum (n=1). ALA were found in the SB (n=7) and rectum (n=2) and often described as incidental yellow-white plaques on endoscopy. Patients most commonly reported abdominal pain (ALO 66%; PLO 58%), were asymptomatic (ALO 33%), or had SB obstruction (PLO 17%). Distant colonic adenocarcinoma was documented in 1 ALA and 3 ALO.

Histologic Findings

	Submucosal involvement	Mucosal involvement	Complete endothelium lined spaces	Surrounding smooth muscle	Surrounding lymphoid tissue	Foam Cells present
PLO	50%	8%	92%	58%	100%	25%
ALO	88%	44%	100%	100%	11%	0%
ALA	90%	100%	9%	0%	9%	0%

Conclusions: This series better characterizes lymphangiomas in the GI tract as symptomatic masses with confluent and distinct lymphatic spaces. Additionally, PLO are larger than ALO (p=0.035) with abundant lymphoid tissue, and can present as SB obstruction. ALA are asymptomatic, have female predominance, and diffusely involve the mucosa and submucosa without a distinct endothelial/smooth muscle lined space. Future studies including immunohistochemistry to further delineate the histologic differentiation of ALO/PLO and ALA, and inclusion of cases from other centers are warranted.

579 Hep Par 1 Expression in Small Intestinal and Colorectal Epithelium and Adenocarcinomas

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Background: Hep Par 1 (hepatocyte antigen) has been considered a relatively sensitive and specific marker of hepatocellular differentiation. It is frequently expressed in hepatocellular carcinomas and tumors with hepatoid differentiation, and only occasionally expressed in nonhepatic neoplasms. Interestingly, Hep Par 1 is also detected in normal small intestine, but whether it is similarly expressed in adenocarcinoma of the small intestine has not been well investigated.

Design: A total of 39 surgically resected primary small intestinal adenocarcinomas (SIAs), including 21 from the jejunum, 7 from the ileum, 5 from the duodenum (distal from the ampulla), and 6 with unspecified site, were immunohistochemically stained for Hep Par 1 antigen using a monoclonal antibody OCH1E5. The stain was considered positive if $\geq$5% of the cells of interest exhibited immunoreactivity. Positive stains were stratified into focal if 5-50% of the cells stained and diffuse if >50%. For comparison, 31 randomly selected sporadic colorectal adenocarcinomas (CRCs) were similarly examined.

Results: Thirty-five SIA cases contained nonneoplastic small intestinal mucosa on the same sections, of which 31 (89%) showed granular cytoplasmic staining for Hep Par 1 exclusively in the epithelium. In contrast, the nonneoplastic colonic epithelium present in all 31 CRC cases was completely negative for Hep Par 1 expression. Nine (23%) SIAs showed positive cytoplasmic staining for Hep Par 1 with a diffuse staining pattern observed in 2 and focal in 7. Although duodenal adenocarcinomas tended to more frequently express Hep Par 1, seen in 3 of 5 cases (60%), a statistical significance was not reached when compared with jejunal (14%; P=0.0624) or ileal (29%; P=0.5581) counterpart. Three (10%) CRCs showed positive Hep Par 1 staining (1 diffuse, 2 focal), a frequency that was not statistically different from that observed in SIAs (P=0.2467). The Hep Par 1 positive tumors were histologically indistinguishable from negative ones and none of the SIAs and CRCs exhibited evidence of hepatoid differentiation.

Conclusions: Hep Par 1 immunoreactivity is selectively expressed in nonneoplastic small intestinal enterocytes but not in colonocytes, suggesting a functional role of Hep Par 1 antigen in intestinal biology. Loss of expression of this antigen in a large number of SIAs suggests a role in small intestinal tumorigenesis, which is apparently distinct from that involved in CRC development.

580 Late Pathological Features in Small Intestinal Allografts

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Background: Small intestinal transplantation is an effective means of treating patients with irreversible small intestinal failure. Its success often depends on the recognition of potential complications in endoscopic biopsies. Although the spectrum of histological findings and clinical correlates in early allografts has been well characterized, that of allografts beyond the first year has not.

Design: We reviewed the pathology reports of all patients cared for at our institution >1y post-transplant whose most recent biopsies had occurred within 2y of the study date. The following predefined parameters were evaluated: acute cellular rejection (ACR), specific viral infection, active (neutrophilic) enteritis (AE) with and without mononuclear (M) or eosinophil (E) infiltrates, inactive chronic enteritis (ICE), apoptotic index, ileal ulcer, transplant vasculopathy (TV) and mesenteric fibrosis. These were correlated with data pertaining to the patients' management and clinical courses. All diagnoses had been issued by expert GI transplant pathologists and reviewed in joint clinicopathological conferences.

Results: 16 patients, 8 adults and 8 children, who met the inclusion criteria had a total 268 biopsies (3-53/patient, mean 17) and 2 explants. Findings ranked by frequency were (# biopsies, % total biopsies): AE-M (73,27%), AE+M (63,24%), ACR (58,22%), ileal ulcers (19,7%), ICE (14,5%), CMV (6,2%), adenovirus (4,1.5%), PTLD (2,1%). AE was usually mild. In biopsies with AE, 20% had elevated apoptotic indices (>6/10 crypt cross-sections) and were diagnosed as ACR; the other 80% were unexplained, showing no correlation with ACR, incipient ACR or infection. Of 55 procedures with AE and concurrent ileal and jejunal allograft biopsies, ileitis was more severe and/or more prevalent than jejunitis (93% v. 45%, P<.00001). In ~50% of 58 procedures with AE and concurrent native bowel biopsies, the latter showed comparable inflammation. AE+E occurred in 35% of children but not in adults (P=.00007). Persistent ileal ulcers occurred in 7 patients (44%) but were usually unexplained. Pre-explant biopsies in 2 patients with TV showed nonspecific changes. One of these explants showed fibromatosis-like mesenteritis.

Conclusions: Many common findings in late small intestinal allografts, i.e., AE, ICE, eosinophilia and ileal ulcers, are idiopathic and do not necessarily portend ACR or other identifiable complications. Eosinophilia appears predominantly in children. Specific infections are relatively uncommon. Biopsies are not a sensitive indictor of impending TV, a major cause of late allograft failure.

581 Collagenous Sprue – A Clinico-Pathological Study of 9 Cases

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Background: Collagenous sprue is a rare form of small bowel enteropathy with little data available on its natural history. The pathologic lesion consists of subepithelial collagen deposition associated with variable alterations in villous architecture. The disease is characterised clinically by chronic diarrhoea and progressive malabsorption.

Design: Cases were retrieved from 5 institutions. Small bowel biopsies were reviewed and morphologic features documented. Clinical details, follow-up data, coeliac serology and T cell receptor rearrangement study results where available were collated.

Results: 9 cases were reviewed. There were 6 females and 3 males (41 to 72 years). Patients presented with chronic diarrhoea, weight loss and hypoalbuminaemia. Small intestinal biopsies showed subepithelial collagen deposition with varying degrees of villous atrophy and varying numbers of intra-epithelial lymphocytes. Two patients had previous biopsies showing enteropathic changes without subepithelial collagen deposition. Six cases were associated with collagenous colitis. Of these 6 patients, 3 also had collagen deposition in gastric biopsies. One case was associated with lymphocytic gastritis. Coeliac serology was available in 6 cases. Serology was negative in 5 cases and positive in 1 case (anti-endomysial antibody). Five patients made a clinical improvement with combinations of a GFD (gluten free diet) and immunosuppressive therapy. Three patients died, 2 of complications of malnutrition and 1 of an intercurrent illness related to previous co-morbidities. Aberrant T cell populations were identified in 4/5 cases tested. Two of these patients improved clinically following treatment and 2 have died of complications of malnutrition.

Conclusions: This relatively large series of collagenous sprue shows female predominance with onset in middle-aged to elderly patients. There is an association with collagen deposition in other parts of the GIT. Coeliac serology is usually negative. Response to GFD and immunosuppressive therapy is variable. Outcome is unpredictable and the role of molecular studies in prognostication needs further investigation.

582 The Effect of Tumor Characteristics and Duplication of the Muscularis Mucosae on Endoscopic Staging in Barrett Esophagus Related Neoplasia

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Background: Duplication of the muscularis mucosae (MM) is a unique property of Barrett esophagus (BE). Misinterpretation of this peculiar pathologic change may lead to inaccurate staging of BE-related intramucosal adenocarcinomas (IMC), particularly in endoscopic mucosal resection specimens (EMRs). Tumor characteristics, architectural changes associated with MM duplication, and expertise of the operator likely converge to affect endoscopic ultrasound (EUS) staging and ultimately may influence the treatment of BE-related IMC.

Design: Our study group consisted of 33 EMRs with a pathologic diagnosis of IMC. Each case was appropriately oriented and contained mucosa, MM, and submucosa. The following features were evaluated: growth pattern and vertical dimension of tumor, thickness of the original MM, presence or absence of MM duplication, prolapse changes and entrapment of glands in the MM. The results were compared with pre-operative EUS staging.
Results: Of the 33 cases, 11 were staged submucosal invasive adenocarcinoma by EUS, whereas the remaining 22 were observed to be confined to the mucosa. The dimension of the tumors was greater in the discordant compared to the concordant cases (1.68 mm vs. 1.22mm, $p<0.05$). The thickness of the original MM was similar between the two groups. Of 11 discordant cases, 7 (64%) had established duplication of the MM and 2 (18%) had a thick single MM layer with prolapse and gland entrapment. The 2 remaining discordant cases had a bulky tumor invading the MM and prominent submucosal glands underlying IMC, respectively. Tumor invaded the MM in 6 (55%) with involvement of single MM in 1, superficial MM in 2, space between both MM layers in 1 and deep MM in 2. In contrast, only 2 (8%) of the concordant cases showed established MM duplication, 5 (23%) had focal poorly formed MM duplication, and 8 (36%) had a single thick MM with prolapse and gland entrapment. An exophytic growth of tumor was seen in 4 (18%) and sole MM invasion was noted in 9 (41%). Among large (≥ 1 mm thick) and non-exophytic tumors, 4 of 8 discordant cases were associated with established MM duplication compared to none of 6 concordant cases.
Conclusions: Duplication of the MM and its related changes, in addition to tumor characteristics (i.e. depth of invasion), can lead to inaccurate EUS staging in BE-related IMC. It is important for pathologists to inform gastroenterologists and surgeons of this critical histopathologic change characteristic of BE.

583 Genetic Markers and Pediatric Onset Inflammatory Bowel Disease

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Background: The diagnosis and classification of inflammatory bowel disease (IBD) can sometimes be difficult. Recently, polymorhphisms/mutations in the NOD2/CARD15 gene, the IBD5 locus and other loci have been shown to be associated with familial ulcerative colitis (UC) and/or Crohn's Disease (CD). We performed this study to determine the frequency of these variants in unselected UC and CD cases and controls.
Design: DNA from formalin fixed paraffin embedded (FFPE) tissue 42 consective cases of pediatric CD, 30 UC, 3 IBD unclassified, and 54age-matched controls (C) was genotyped for variants in NOD2 (G908R, IVS8+158C>T, 3020insC, R702W and); IBD5 locus (IGR2055T>G, IGR2060G>C, IGR2063G>C, IGR2078A>G, IGR2096A>C, IGR2198G>C, IGR2230T>C, IGR2277A>G, IGR3096T>C, IGR3236T>G, IGR3081T>G); NFKBIA (1176A>G); TNF (1031T>C; 238G>A; 308G>A and 857C>T), using a Sequenom array mass-spectroscopy, in two separate reactions. The genotypes of cases and controls were compared with a two-tailed Fisher's Exact Test.
Results: A homozygous IBD5 haplyotype (IGR2055G, IGR2060C, IGR2063C, IGR2078A, IGR2096A, IGR2198G) spanning approximately 20kb was seen in 11/40 CD 3/29 UC, 0/3 IBDU and 2/53 C (odds ratio [OR] for CD vs C: 9.7, p=.0017; OR for IBD vs C: 4.35 p = 0.013). Heterozygosity or homozygosity for the CARD15 IVS+158T allele was seen in 13/29 UC, 9/42CD 0/3 IBDU and 8/51 C (OR for IBD vs C: 2.27 p>.05, p=; OR for UC vs C: 4.88 p<0.01). The remaining variants were too infrequent for statistical analysis. No definite relationship with clinical characteristics was seen.
Conclusions: Our results show the feasibilty of high throughput genotyping using residual material and array mass spectroscopy. In addition they show the frequency of homozygosity of the IBD5 high risk haplotype in pediatric CD (11/40 or 27.5%, with an OR of 9.7), and heterozygosity or homozygosity for the the CARD15 IVS8 polymorphism in UC (13/29 or almost 45% UC and associated with an OR of 4.88). The frequency of these variants in an unselected population suggests that testing for these polymorphisms may be of value in the work-up of suspected pediatric IBD.

584 Clinicopathologic Features and Prognosis of High-Grade Neuroendocrine Carcinoma of Esophagus

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Background: Small cell carcinoma (SCC) of esophagus has an aggressive behavior. The literature on esophageal neuroendocrine carcinoma (NEC) other than SCC is limited. We evaluated clinicopathologic features and prognosis of high-grade NEC of esophagus.
Design: Institutional database (1996-2006) was searched to identify thirty-seven patients of esophageal high-grade NEC. All patients were clinically staged with endoscopic ultrasonography with fine needle aspiration of suspicious regional lymph nodes, CT and PET scan. The neuroendocrine differentiation was confirmed by immunohistochemistry for chromogranin and synaptophysin. Neuroendocrine component was classified into small cell and large cell utilizing the criteria used in classification of neuroendocrine tumors of lung with focus on tumor necrosis, nuclear features, nucleolar prominence and easily identifiable mitosis. All patients were followed with imaging studies, endoscopy ultrasound and biopsy of suspicious lesions. Presence and extent of residual tumor was evaluated in esophagectomy specimen performed after chemoradiation. The chemotherapy consisted of platinum based regimens.
Results: Twenty-four (65%) patients had a large cell NEC and 13 (35%) had SCC. Adenocarcinoma component was present in 13 (35%) patients (11 with large cell NEC and 2 with SCC) and 1(3%) SCC had squamous carcinoma component. Synaptophysin was positive in all cases and chromogranin was positive in 31. Eighteen (49%) patients had systemic disease (stage IVB) and 19 (51%) had loco-regional disease (5 stage IVA, 9 stage III, 4 stage II, 1 stage I). Fourteen patients with loco-regional disease underwent preoperative chemoradiation. Five patients had progression on treatment with distant metastasis and 9 patients underwent esophagogastrectomy after completion of chemoradiation. All 9 patients had residual disease with more than 50% viable tumor on histopathologic assessment of resected specimen. Median survival for patients with systemic disease was 11.56 ± 2.42 months and 39.46 ± 12.12 months for patients with locoregional disease ($p<0.01$). No survival difference was noted between large cell NEC and SCC.
Conclusions: High-grade NEC of esophagus is an aggressive disease and has higher stage at presentation with poor response to preoperative chemoradiation.

585 Frequent Loss of Heterozygosity (LOH) of Chromosome 1q in Esophageal Adenocarcinoma

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Background: Previous microarray expression profiling studies have shown that genes located on chromosome 1q21 region are down regulated in progression of Barrett's esophagus to adenocarcinoma and are differentially expressed between responders and non responders of preoperative chemoradiation in esophageal carcinoma. It is unclear whether this is due to loss of heterozygosty (LOH) at this locus or methylation of genes at this locus. Present study evaluated the status of 1q LOH in esophageal adenocarcinoma specimens and correlated with pathologic response and overall survival.
Design: Pretreatment biopsies of twenty four patients with esophageal adenocarcinoma who underwent preoperative chemoradiation followed by esophagectomy were evaluated for 1qLOH. LOH status was determined by PCR analysis of 9 dinucleotide microsatellite markers that spanned 1q21-23 region using fluorescent dye-labeled (6-FAM, VIC, or NED) primers (Applied Biosystems, Foster City, CA or Invitrogen, Carlsbad, CA) and capillary electrophoresis. DNA was retrieved following manual microdissection of formalin fixed paraffin embedded tissue performed by one pathologist (DM). Loss of a marker was considered to be present when there was absence or decrease in intensity by more than 50% of one of the two alleles from a tumor sample as compared with the paired non-neoplastic squamous mucosa. The 1q LOH status of each marker was correlated with the status of residual tumor and overall survival.
Results: As shown in table 1 and 2 LOH at 1q21, 1q21.2, 1q21.3, 1q23.1, 1q23.2 and 1q23.3 was noted with higher frequency with more than half of cases for each locus with informative markers showing LOH.

Table 1

Chromosomal location	1q21	1q21.2	1q21.3	1q21.3
Marker	D1S2125	D1S3466	D1S498	D1S2343
Monoallelic (n)	15	9	10	10
Polymorphic without LOH (n)	6	8	4	4
LOH (n)	3	7	10	10

Table 2

Chromosomal location	1q21.3	1q23.3	1q23.2	1q23.1	1q23.2
Marker	D1S2715	D1S484	D1S2635	D1S2624	D1S1167
Monoallelic (n)	5	15	12	16	6
Polymorphic without LOH (n)	13	4	5	3	12
LOH (n)	6	5	7	5	6

All three cases with LOH at D1S2125 showed complete pathologic response. However, no significant correlation was identified with 1q LOH and overall survival and LOH at other loci with pathologic response.
Conclusions: 1q21 LOH is a frequent event in esophageal adenocarcinoma and thus may explain previously observed down regulation of genes at this locus. This region should be explored more in depth to evaluate the role of LOH with response to preoperative chemoradiation in esophgeal adenocarcinoma.

586 True Smooth Muscle Tumors of the Jejunum and Ileum – A Clinicopathologic Study of 32 Cases

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Background: Most small intestinal mesenchymal tumors are GISTs, but a small group of true smooth muscle tumors exist. Their clinicopathologic profile and KIT/PDGFRA mutation status has not been sufficiently analyzed.
Design: We analyzed > 1200 mesenchymal tumors of the jejunum and ileum originally identified as smooth muscle tumors. Among these, 48 true smooth muscle tumors were found, including 29 tumors that were primary to these sites. These tumors were positive for SMA, desmin, and heavy caldesmon, and were negative for KIT. These tumors were analyzed clinicopathologically, and for KIT/PDGFRA mutation status for regions known to be involved in GISTs.
Results: The 29 tumors occurred in 16 males and 13 females with median age of 63 years. There were 10 patients with well-differentiated smooth muscle tumors with mitotic activity <5/50 HPFs. These tumors were considered intramural leiomyomas (LMs) except for one LM of the muscularis mucosae. Tumor size ranged 0.8-9 cm. Five of these patients were verified to be free of disease (median follow-up, 16 years), and there were no patients with metastasis or tumor death. There were 19 well-differentiated smooth muscle tumors that contained mitotic activity > 5/50 HPFs, all but one having >30 mitoses/50 HPFs. These tumors (size 3-29 cm) were considered leiomyosarcomas (LMSs). Seven patients developed metastases or died in <5 years, whereas 4 patients survived 10 or more years although died of unknown causes. The tumor size of the survivors ranged from 3-9 cm. One patient whose 9 cm tumor had 6 mitoses/50 HPFs had 3 recurrences in 15 years. All six leiomyosarcomas analyzed had wild-type KIT and PDGFRA sequences.
Conclusions: True smooth muscle tumors are rare in jejunum and ileum, and comprise no more than 2.5% of tumors originally considered such. These tumors (SMA/Des +, KIT -) seggragate well into mitotically inactive LMs and mitotically highly active LMSs; all tumors with mitotic rate <5/50 HPFs had uneventful follow-up. Mitotically active tumors (usually high mitotic rate) had a variable behavior, with many patients developing metastases, but some surviving for long periods of time; tumor size may be a prognostic

factor. There is no evidence that morphologically and immunohistochemically defined true smooth muscle tumors are KIT-negative GISTs by their negative KIT/PDGFRA mutation status.

587 Immunohistochemical Classification of Primary Gastrointestinal Diffuse Large B-Cell Lymphomas

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Background: The gastrointestinal tract (GIT) is the most common extranodal site of lymphoma, and the most common lymphoma in the GIT is diffuse large B-cell type (DLBCL). Studies have indicated that prognosis can be predicted by classifying DLBCL into germinal center (GCP) and non-germinal center (non-GCP) phenotypes using CD10, BCL-6 and MUM1 immunohistochemistry (IHC), with a better prognosis reported in GCP. Most prior studies have been performed on non-GIT lymphomas, with less extensive analysis of GIT DLBCL. Therefore, we classified primary GIT DLBCL at the University of Pittsburgh Medical Center (UPMC) using patterns of immunostaining, and compared gastric and intestinal subgroups.

Design: 51 GIT DLBCL diagnosed between January 1995 and June 2007 at UPMC were retrieved. Cases with a prior diagnosis of lymphoma outside the GIT, coexisting non-contiguous nodal lymphoma or on which the diagnosis was not confirmed were excluded. Relevant demographic and clinical data were obtained for each case and each was evaluated with CD10, MUM1 and BCL-6 IHC. All gastric lymphomas were also stained for *Helicobacter pylori* (HPYL). The lymphomas were grouped into GCP and non-GCP based on a previously-published algorithm and the results were analyzed according to site of involvement and phenotype.

Results: There were 25 gastric DLBCL, 18 small intestinal, 6 colonic and 2 spanning the ileocecal valve. There were 24 women and 27 men, with age range 27-102 (mean=74). 37 (73%) cases were GCP and 14 (27%) non–GCP ($p = 0.001$). Mean age of GCP was 75 and of non-GCP 71 ($p = 0.26$). GCP was seen disproportionately in the intestines, and non-GCP in the stomach ($p = 0.01$). HPYL was positive in 9/24 (38%) primary gastric DLBCL (4 GCP and 5 non-GCP; $p = 0.68$).

Conclusions: Primary GIT DLBCL were most often gastric (49%), followed by small intestinal. Colonic DLBCL were uncommon, and there were none in the esophagus or anus. The difference in phenotype between the stomach and intestines may reflect origins of GIT DLBCL from either acquired or native mucosa-associated lymphoid tissue (MALT), while the lack of a significant difference in HPYL between GCP and non-GCP may result from the heterogeneity of HPYL-induced MALT in the stomach. Alternatively, some gastric DLBCL may arise without a pre-existing MALT lymphoma. The expected prognostic advantage of GCP DLBCL in the intestines may be thwarted by presentation at a higher stage than in the stomach, where ulcer/GIT bleed is a more common presentation.

588 Diagnostic Utility of WT1 Immunostaining in Gastrointestinal Stromal Tumor

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Background: Immunohistochemical detection of c-kit expression is considered a defining diagnostic test for gastrointestinal stromal tumor (GIST). However, c-kit expression is not entirely GIST-specific. One of the examples is intra-abdominal fibromatosis (IAF), which can morphologically and immunophenotypically mimic GIST. The distinction between these two neoplasms is important because of their different biological behavior and management. It has been shown that the Wilms' tumor gene product, WT1, is expressed in various tumors, but its expression has not been examined in GISTs and IAFs.

Design: Surgically resected or biopsied GISTs (n=51) and IAFs (n=11) were immunohistochemically studied, using antibodies against to c-kit (Dako) and WT1 (Dako). Cytoplasmic staining for both proteins was considered positive. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as weak, moderate, or strong.

Results: All 51 GISTs (100%) and 8 IAFs (73%) were positively stained for c-kit, albeit with variable intensity and extent. Interestingly, WT1 was also detected in all GISTs including 43 cases (84%) showing a diffuse and moderate to strong cytoplasmic staining pattern. The remaining 8 cases exhibited focal (<10% of the tumor cells stained) and/or weak cytoplasmic positivity. In contrast, none of the 11 IAFs expressed WT1, including those showing a strong c-kit immunoreactivity.

Conclusions: WT1 immunostaining may serve as a supplementary marker for the diagnosis of GIST, which may be particularly useful in the segregation between GIST and IAF. The mechanism of cytoplasmic localization of WT1 in GIST and its biological role in GIST tumorigenesis warrant further investigation.

589 Frequent CpG Methylation of Thrombospondin-1 in Colonic Adenomas and Adenocarcinomas

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Background: Decreased expression of thrombospondin-1 (THBS1), an angiogenesis inhibitor, occurs in some human tumors. Loss of THBS1 was reported in colonic adenomas and in invasive colon cancers. CpG island methylation may be a mechanism of epigenetic gene inactivation of THSB1 in colorectal carcinogenesis.

Design: We studied 37 colonic neoplasms (21 adenocarcinomas and 16 adenomas) and the background normal mucosa in 34 cases. Normal colonic mucosa of 10 patients without colonic neoplasms was used as control. The mean ages of patients with adenomas, adenocarcinomas or controls were 67, 64, and 53 years, respectively. The methylation status of CpG islands in the promoter region of the THBS1 gene was evaluated by methylation specific PCR (MSP) of bisulfite modified DNA.

Results: THBS1 CpG methylation was more frequent in carcinomas (17 of 21, 81%) and adenomas (12 of 16, 75%) as compared to normal colonic mucosa of patients without colonic neoplasms (zero of 10, 0%) ($P<0.001$). Methylation of THBS1 was detected in most adenomas (75%) and carcinomas (81%), and in a smaller number of mucosal tissues adjacent to colonic carcinoma (3 of 18, 17%) or adenoma (2 of 16, 13%), ($P<0.001$).

Conclusions: The THBS1 CpG promoter islands are methylated in most colonic adenomas and adenocarcinomas, but rarely in the non-neoplastic colonic mucosa of the same patients, suggesting a possible role of THBS1 in colonic carcinogenesis. Epigenetic regulation of this gene appears to occur early in colonic neoplasia since CpG methylation was similarly frequent in adenomas and carcinomas.

590 Pathology of Late Graft Failure in Small Bowel Transplantation

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Background: Small bowel transplantation has been performed at a limited number of centers. The rate of graft failure is still high, largely due to allograft rejection and infections. Only a few studies have been performed concerning the pathology of small bowel allograft and the histologic features of late graft failure are not yet well-defined. This study aims to characterize the features that would help to histologically define late graft failure and to compare these features to those in graft failure of non otherwise-specified. The results will aid in identifying reasons for graft failure.

Design: We performed a retrospective study of all small bowel transplant recipients at UCLA (1994-2007) who had graft failure and underwent surgical partial or total enterectomy. Twelve cases were identified, of which four lost the graft after 100 days (i.e. late graft failure), and the remaining eight lost the graft before 100 days. We performed a comprehensive histological exam evaluating for all the standard/identifiable pathological changes observed in mucosa, submucosa, muscularis propria and serosa/vessels.

Results: The following histological findings were present in equal or greater than 50% of cases: mucosal ulceration (9/12), epithelial injury/repair (8/12), undulating/wavi- form architecture (6/12), architectural distortion (9/12), crypt hyperplasia (9/12), villous blunting (9/12), apoptosis >6 in 10 consecutive crypts (6/12), mucosal ischemic necrosis (6/12), organizing serositis (9/12), and serosal fibrosis (6/12). The frequency of most characteristic findings compared in late graft failure cases to all cases as follows: mucosal ulceration (75% in both groups); undulating architecture (50% in both groups); epithelial injury/repair (50% of late graft failures, 67% of all cases), submucosal fibrosis (25% of late graft failures, 17% of all cases); muscularis propria fibrosis and myocyte dropout (none of late graft failures, 33% of all cases); organizing serositis (75% of both groups); obliterative arteriopathy (25% of late graft failures, 8% of total cases).

Conclusions: The majority of histologic features of late graft failure are non-specific, making accurate histologic diagnosis very challenging. However, frequent features include discrete mucosal ulceration with mucosa showing undulating architecture and evidence of injury/repair. An infrequent but characteristic feature is obliterative arteriopathy of large arteries within serosa which is analogous to those seen in late graft failures of other solid organs.

591 Heparanase Induction in Barrett's Mucosa and during Esophageal Carcinoma Progression

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Background: Heparanase is an endoglycosidase that specifically cleaves heparan sulfate (HS) side chains of HS proteoglycans, its activity is strongly implicated in cell dissemination, associated with tumor metastasis, inflammation and angiogenesis. Heparanase up-regulation was documented in an increasing number of human carcinomas and hematological malignancies, correlated with reduced postoperative survival rate. This study was undertaken to investigate the involvement of heparanase in the progression of esophageal carcinoma.

Design: The study included samples from 66 patients: 11 normal, 21 Barrett's mucosa (BM) without dysplasia, 5 BM with low grade dysplasia, 12 BM with high grade dysplasia and 17 adenocarcinomas. Paraffin sections were stained with anti-heparanase antibodies and heparanase staining localization, intensity and extent were correlated to pathological diagnosis and proliferation index revealed by anti-Ki-67 staining.

Results: Normal mucosa stained negative for heparanase. Positive staining of heparanase was observed in 52% (11/21) of the cases with BM without dysplasia, increase that is statistically highly significant ($p=0.0045$). Statistically significant increase in heparanase staining was also found between BM without dysplasia and BM with high grade dysplasia ($p=0.005$). All cases of high grade dysplasia and carcinoma stained positive. However, only 33% cases (4/12) of high grade dysplasia exhibited strong staining of heparanase compared with 88% (15/17) of carcinoma cases exhibiting such staining ($p=0.0045$). Differences were also noted in heparanase localization. Thus, while BM exhibited cytoplasmic staining of heparanase, 70.6% (12/17) of the carcinoma cases exhibited also nuclear staining, compared with one case of high grade dysplasia exhibiting such staining pattern ($p=0.0018$). Moreover, heparanase staining correlated with cell proliferation revealed by staining for Ki-67 ($p=0.0006$).

Conclusions: Heparanase induction occurred already at BM without dysplasia.Further increase in heparanase staining intensity and extent was found in the progression from low to high grade dysplasia and adenocarcinoma. These results suggest that heparanase plays a role at the early stages of esophageal carcinogenesis, and imply that the enzyme is a valid target for the development of anti-cancer drugs.

592 FISH for DCC Gene Deletion in Colorectal Cancer

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Background: Deleted in colon cancer (DCC) gene is a putative tumor suppressor gene located at 18q21.2 that is involved in allelic deletion in 50% to 70% of colorectal cancers

(CRCs). Allelic loss of 18q in primary CRC is associated with an increased death rate independent of stage and clinical and pathologic features and a possible predictor of response to FU-based chemotherapy. The primary means of determining loss of 18q have traditionally been by loss of heterorzygosity (LOH) analysis using a panel of markers that are 5 to 10 Mb base pairs telomeric to the DCC gene.
Design: Twelve consecutive cases of colorectal carcinoma were evaluated for LOH at 18q21 using a panel of microsatellite markers and deletion of the DCC gene by FISH. The microsatellite markers included the widely publicized set of D18S69, D18S64, D18S55, D18S61 and D18S58 done using both a singleplex and multiplex approach. FISH for DCC deletion was performed using 6 separate BAC clones (RP11-7K9, RP11-104G8, RP11-869O1, RP11-182L8, RP11-751P21, RP11-671P2; Spectrum Orange) with a CEP18 probe (Spectrum Green). Frozen tissue was available for all specimens.
Results: LOH was identified with one or more microsatellite markers in 8 of 12 (75%) of cases. FISH results were available for 11 of 12 cases, including all cases with LOH. For those cases with no LOH, there was no deletion of DCC or monosomy for chromosome 18 by FISH. For 3 of 8 cases with LOH, there was monosomy 18 and no homozygous deletion of DCC. Of the remaining 5 cases with LOH, 2 showed DCC deletion and 3 did not.
Conclusions: Our number of samples is currently too small for statistical evaluation. As expected, all cases with monosomy 18 showed haploinsufficiency for all LOH markers. Those cases with LOH but no DCC deletion or monosomy 18 may be the result of loss of one copy of chromosome 18 with subsequent duplication of the remaining copy. A plausible alternative is the common deletion at 18q21 in CRC is telomeric of the DCC gene with possible implications of a different gene involved.

593 Selective Expression of Gastric Mucin MUC6 in Colonic Sessile Serrated Adenoma but Not in Hyperplastic Polyp Aids in Morphological Diagnosis

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Background: Sessile serrated adenoma (SSA), while lacking cytologic dysplasia, is thought to be related to sporadic microsatellite-unstable colon cancer by the "serrated neoplasia" pathway, involving loss of expression of MLH-1. In contrast to SSA, hyperplastic polyp (HP) is essentially devoid of cancer risk, so accurate distinction between SSA and HP is important. However, the diagnosis of SSA relies on architectural features that are subject to interobserver variability and more objective and reproducible criteria are desirable. We studied the expression of MLH-1 and MUC6, a gastric type mucin, in a group of serrated polyps previously reported to exhibit gastric differentiation in the literature.
Design: 84 serrated polyps were randomly collected during clinical service at our institution. They were assessed for architectural features (dilation, branching, serration and horizontality of basal crypts) and classified as SSA (at least 2 features present) or HP, with their location in the colon noted as right (RC; proximal to splenic flexure) or left (LC). 5 traditional serrated adenomas (TSA) and 5 SSA with areas of cytologic dysplasia (SSAD) were also included. All polyps were stained with immunohistochemical antibodies to MUC6 and MLH-1.
Results: The polyp subtypes were similar in size, from 2-18 mm. 21/26 (81%) SSA were found in the RC and 47/48 (98%) HP in the LC. All TSA were from the LC and all SSAD were from the RC. All SSA and SSAD were positive for MUC6, with staining restricted to basal crypts, while HP and TSA were negative. All HP, SSA and TSA retained MLH-1 expression, while 4/5 SSAD were negative for MLH-1 in the cytologically dysplastic areas (which also showed MUC6 staining).
Conclusions: Selective expression of MUC6 in SSA and SSAD, but not HP or TSA, is a potentially useful adjunct in the diagnosis of SSA. Loss of MLH-1 is a late event in the pathogenesis of SSA associated with cytological dysplasia, while ectopic expression of MUC6 is an early event associated with characteristic architectural features in basal crypts. The coexisting loss of MLH-1 and expression of MUC6 in cytologically dysplastic areas of SSAD suggests that MUC6 plays an important role in the malignant transformation of SSA. Neither anatomical location nor polyp size seem to account for the differences in MUC6 staining (5 polyps with features of SSA were found in the LC and all were positive for MUC6).

MUC6 and MLH-1 In Serrated Polyps

	SSAD	SSA	HP	TSA
Number	5	26	48	5
MLH-1(+)	1	26	48	5
MUC6(+)	5	26	0	0

594 Thymidylate Synthase Expression in Pseudomyxoma Peritonei Correlates with the Grade of Malignancy

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Background: Pseudomyxoma peritonei (PMP) is a rare disease characterized by peritoneal spread of a mucinous neoplasm, most commonly of appendiceal origin, and massive accumulation of mucin in the abdomen. There are generally two histologic and clinical types of mucinous carcinomatosis: low grade (disseminated peritoneal adenomucinosis, DPAM) and high grade (peritoneal mucinous carcinomatosis, PMCA), which are associated with low grade mucinous appendiceal neoplasms and appendiceal mucinous carcinomas, respectively. Expression of thymidylate synthase (TS) is an important prognostic marker of tumor sensitivity to 5-fluorouracil (5-FU) based chemotherapy, which is an essential part of treatment regime for pseudomyxoma peritonei. We evaluated the expression of TS in PMP and its correlation with tumor grade, proliferative fraction and expression of p53.
Design: 62 patients with PMP were studied, including 28 men and 34 women with a mean age of 57.8 years and 54.4 years respectively. Protein expressions of TS, Ki67 and p53 were performed using standard immunohistochemical techniques, and all specimens were evaluated by two pathologists. The expression of TS was scored using histo-score (H-score) incorporating the intensity (I) of staining (0-3) and proportion (P) of cells (0-1.0) expressing the protein (H=I×P).
Results: 35 patients with PMP were classified as low grade (DPAM), and 27 were classified as high grade (PMCA, including intermediate cases). In general, the expressions of TS, Ki67 and p53 correlated with the grade of PMP, and the difference in TS expression between DPAM and PMCA was statistically significant ($p<0.04$). Ki67 expression correlated with p53 and TS expressions irrespective of PMP type ($p<0.05$), and TS expression also correlated with p53 expression, although it was not statistically significant ($p>0.05$).
Conclusions: The protein expression of TS is significantly higher in the high grade PMP than in the low grade PMP, and TS expression correlates with p53 and especially Ki67. These findings are consistent with the TS data on colorectal cancers. TS can be considered as a prognostic marker in management of PMP, and patients with high TS levels may benefit from adjuvant 5-FU–based chemotherapy. We are currently correlating polymorphisms of TS gene with its protein expression in PMP in order to better understand the mechanisms of TS expression and its significance in PMP.

595 Cox-2 Is a Valuable Prognostic Marker in Gastric Carcinoma and Is Not Associated with EBV Infection

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Background: Cyclooxygenase-2 (Cox-2) is believed to be involved in carcinogenesis and recent study proved that an elevated risk of gastric carcinoma (GC) was observed in subjects carrying AA genotype and *H pylori* infection. *H. pylori* induces Epstein-Barr virus (EBV) reactivation in gastric epithelium latently infected with EBV. EBV is detected in about 10% of gastric carcinoma throughout the world and thought to be related to the pathogenesis of GC. But there are insufficient data to prove that EBV infection is directly involved in GCs.
Design: Microarray tissue samples from 457 GC patients who underwent gastrectomy were studied with EBER1 in situ hybridization for EBV and immunohistochemistry for EBV-related proteins (hMLH1, E-cadherin, c-erbB, GSTPi and Cyclin D-1). The data was analyzed with χ^2 or Fisher's exact test and their relationship to overall and disease-free survival were analyzed by Log rank test or Breslow test.
Results: EBV infection was observed in 10.9% of GCs. Among the proteins, only hMLH1 was associated with EBV infection (p=0.015). Positive expression of Cox-2 (high intensity and >50% of tumor cells) was closely associated with intestinal histologic type, older age (>60), increased expression of c-erbB, hMLH1, GSTPi and E-cadherin ($p<0.0001$). Univariate analysis showed that pT stage ($p<0.0001$) and pN stage (p=0.0043) were significant prognostic factors. The Cox proportional hazard regression analysis indicated that Cox-2 expression (p=0.016, hazard ratio 1.675, 95%CI = 1.099~2.551), pN stage (p=0.001), and resection margin less than 1cm (p=0.006) were independent prognostic factors. Moreover, expression of Cox-2 was closely associated with disease-free survival (p=0.008, hazard ratio 1.749, 95%CI = 1.157~2.643) in addition to pN stages (p=0.003) and resection margin less than 1cm (p=0.031). EBV infection and related altered expression of hMLH1 was not significant prognostic marker in our large scaled study.
Conclusions: Overexpression of Cox-2 is a valuable prognostic marker in both overall and disease-free survival in GCs. EBV infection is not associated with Cox-2 expression or survival of GC. Our results indicate that Cox-2 may play a key role in the development of GC regardless of EBV infection and is closely associated with histologic differentiation and prognosis of GC.

596 A Large Cohort of 270 Eosinophilic Esophagitis Cases – A Single Institutional Study of Distinct Morphologic Features, Classification Consideration and Clinical Correlation

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Background: Eosinophilic esophagitis (EE) is an increasingly recognized esophageal disorder that has emerged as one of the most common causes of dysphagia in adults. The presence of ≥15 eosinophils /hpf (eos/hpf) in an esophageal biopsy is strongly suggestive of EE. However, there is no study on a large cohort of patients analyzing the morphologic features, morphology /etiology-related consideration in its classification, and correlation with diet elimination regimen and anti-reflux therapy.
Design: Our aim was a) to evaluate morphological features in proximal vs distal biopsies, b) compare these parameters between distal-only EE and reflux esophagitis (RE, n=20, controls) and c) evaluate effect of 6-food elimination therapy in 10 patients non-responsive to anti-reflux PPI Rx (ongoing clinical trial). The diagnostic criterion for EE was at least 1 biopsy fragment showed 15 eos/hpf in ≥2 fields. In addition, age, basal cell hyperplasia (BCH) (25%- normal, mild: 26-50%, moderate: 51-75%, severe >75%), spongiosis, ballooning change (focal/diffuse) and max.number of eos/hpf.
Results: We performed a 3-year review of 409 biopsies from 270 patients (186 males, 84 females). Increasing eosinophil nos.were associated with increasing severity of BCH and spongiosis ($p<0.001$ and $p<0.001$). BCH and spongiosis was more prominent distally than proximally ($p<0.001$); BCH was pronounced in cases with involvement of both proximal and distal esophagus (diffuse pattern EE, n=188) when compared to distal-predominant EE (n=82). Diffuse squamous epithelial ballooning change was noted in >50% cases with RE and 12% distal-predominant EE cases ($p<0.001$). Nearly 7% of diffuse pattern EE cases showed ballooning change. Of the10 cases of diffuse pattern EE enrolled in dietary elimination trial, 8 showed a significant improvement in endoscopic findings, eosinophilic infiltrate and spongiosis post Rx (proximal $p<0.005$, distal $p<0.05$).
Conclusions: Increased burden of eosinophils is directly proportional to degree of epithelial injury and reactive hyperplasia (BCH), irrespective of the location. Ballooning

change is a key histological feature that distinguishes RE from distal-predominant EE. Diet elimination trial results suggest that food allergy is a possible etiology for diffuse esophageal disease while reflux appears to be a mediator of distal-predominant EE.

597 High Level of Nucleolin Expression Is Associated with Better Prognosis in Patients with Stage II Pancreatic Ductal Adenocarcinoma

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Background: Nucleolin, a major nucleolar phosphoprotein, interacts with telomerase and alters its subcellular localization through interactions with its RNA binding domain 4 and carboxyl-terminal RGG domain. The interaction between nucleolin and hTERT is critical for the intracellular localization and functions of telomerase complex. The expression of nucleolin and its prognostic significance in pancreatic ductal adenocarcinoma has not been studied. In this study, we examined the expression of nucleolin in 63 patients with stage II pancreatic ductal adenocarcinoma (PDA) and correlated expression with clinicopathologic features.

Design: We used a tissue microarray consisting of duplicate 1 mm cores of tumor and paired benign pancreatic tissue from 63 Whipple's resection specimens for stage II PDA (22 stage IIA and 41 stage IIB). The expression levels of nucleolin were evaluated by immunohistochemistry with a mouse anti-nucleolin monoclonal antibody (Abcam, Inc, Cambridge, MA). The staining results were scored quantitatively using Ariol Image Analysis System (San Jose, CA). Nucleolin expression in PDAs was classified as nucleolin -high or nucleolin -low (nucleolin labeling index >2.8% or <2.8%, respectively) and evaluated for correlation with clinicopathologic features and survival. Statistical analyses were performed using SPSS software (version 12.0; SPSS, Chicago, IL).

Results: Nucleolin expression level was higher in PDAs than in non-neoplastic pancreatic ducts. Nucleolin showed a nucleolar staining pattern in PDA. Among the 63 PDAs, 30 (48%) were nucleolin-high. Nucleolin-high was associated with better overall survival. The median overall survival was 42.6 ±16.9 months for patients with nucleolin-high tumors and 18.9 ± 2.2 months for those patients with nucleolin-low tumors (log-rank test, p=0.03). No significant correlation between the nucleolin expression and other clinicopathologic parameters was found.

Conclusions: Our results indicate that high level of nucleolar expression of nucleolin was present in a subset of patients with stage II PDA and was associated with better overall survival.

598 Post Transcriptional Regulation of Cellular Prion Protein in Pancreatic and Gastric Adenocarcinomas

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Background: The protein over-expression of cellular prior protein (PrP^{C}) has recently been reported in many types of tumor cells, including gastric, breast and pancreas cancers. PrP^{C} is a glycosyl-phosphatidylinositol anchored membrane protein, which can transport copper, function as an adhesion molecule, regulate apoptosis or can be involved in signal transductional pathway. The mutated scrabie form (PrP^{SC}) is responsible for prion disease. Human PrP^{C} located on chromosome 20, and its protein contains 253 amino acids with molecular weight of 33-35 kDa. In this study, we evaluated the relationships of genomic DNA, mRNA and protein levels of PrP^{C} in gastric and pancreatic carcinomas, and to determine at which level the PrP^{C}expression was regulated.

Design: Forty cases of fresh tumors of primary pancreatic and 10 cases of gastric carcinomas were included in this study (IRB approved). Normal gastric (n=5) and pancreatic (n=5) tissues were used as controls. Genomic DNA and mRNA were prepared by DNA and mRNA isolation kit, respectively (Qiagen). Protein levels were examined by Western blot on fresh tissue and immunohistochemistry on fixed samples by using monoclonal anti-PrP^{C}. DNA and mRNA levels were determined by real-time PCR and RT-PCR.

Results: All normal pancreatic and gastric tissue had detectable genomic DNA, mRNA but not protein expression by immunohistochemical stain. Compared to normal tissues, no discernable increase of genomic DNA levels of PrP^{C} in tumors from both pancreas and stomach was observed. Only 15% (6/40) and 10% (1/10) of pancreatic and gastric cancers showed significant increase (> 4 fold) of PrP^{C} mRNA levels. However, 57.5% (23/40) of pancreatic and 40% (4/10) of gastric cancers showed positive expression of PrP^{C} by immunohistochemistry. The protein expressions were confirmed by Western-blot showing the bands in the region of 33-35 kDa.

Conclusions: Our data indicate that there was no significant genomic alteration and only slight transcriptional up regulation of PrP^{C} mRNA in pancreatic and gastric cancers. However, there was a significant up-regulation of PrP^{C} protein expression in these tumors, indicating that the increased expressions of PrP^{C} in these tumors were most likely due to post-transcriptional regulation. Further study will focus on if it is translational or post-translational regulation.

599 Adenoma-Infiltrating Lymphocytes Are a Potential Marker of HNPCC

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Background: Patients with hereditary nonpolyposis colorectal cancer (HNPCC) syndrome develop tumors with high-frequency microsatellite instability that tend to be located on the right side and histologically show high numbers of tumor-infiltrating lymphocytes, a lack of dirty necrosis, a Crohn's-like host response and mucinous and/or poor differentiation, when compared to microsatellite-stable cancers. However, histologic features that are characteristic of and can perhaps distinguish colorectal adenomas in HNPCC patients from non-syndromic patients have not been reported. We undertook this study to see if there are morphologic predictors of adenomas in HNPCC.

Design: H&E-stained sections from 26 colorectal adenomas occurring in patients with genetically-proven HNPCC syndrome were compared with 26 control adenomas that were matched for patient age and sex, and endoscopic size, shape, and anatomic location of the polyp. Three gastrointestinal pathologists blindly evaluated the degree of dysplasia, the percent of villous component, the presence of necrosis and serrated architecture, as well as the number of adenoma-infiltrating lymphocytes (AILs), mitotic figures and apoptotic bodies in these biopsies. The numbers of AILs, mitotic figures and apoptotic bodies were semiquantitatively graded as few (<5/high-power field {HPF; 400x on an Olympus BX40 microscope, total area equal to 0.94 mm^2}), intermediate (5-10/HPF), or high (>10/HPF).

Results: Adenomas from HNPCC patients were more likely to contain areas of high grade dysplasia (35% of cases vs. 4% in the control group; p=0.003). They were also more likely to contain intermediate or high numbers of AILs: 27% and 54% of cases, respectively, compared to 8% and 12% in the control group (p<0.001). Using a cut-off of 5 or more AILs/HPF yielded a sensitivity of 80.8%, a specificity of 80.8%, and a positive predictive value of 80.8% in terms of identifying patients with HNPCC in this study. Necrosis, serrated architecture, percent villous component, and numbers of mitoses or apoptoses did not differ significantly between the two groups.

Conclusions: AILs in colorectal biopsies are a simple and inexpensive marker that, when increased, raise the possibility that the patient has HNPCC. While this is a promising finding, it will need to be tested in a large, population-based study.

600 An Immunohistochemical Panel Distinguishes Colonization by Pancreatic Ductal Adenocarcinoma from Adenomas of Ampullary and Duodenal Mucosa

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Background: Pancreatic ductal adenocarcinoma (PDA) often colonizes the overlying mucosa of the duodenum or ampulla of Vater and, in doing so, acquires histologic features making it difficult to distinguish from a primary adenoma by endoscopic biopsy. Adenomas of the duodenum and ampulla are far less aggressive than PDA and may be treated by polypectomy. The objective of this study was to identify antibodies helpful in differentiating colonizing PDA from adenomas in the duodenal and ampullary mucosa.

Design: Sixty eight resection specimens were initially stained with a panel of antibodies (CK7, CK20, CDX2, MUC1, MUC2, DPC4, p53, and Ki67): 23 cases of PDA with documented colonization of the duodenal mucosa, 15 duodenal neoplasms (14 adenocarcinomas with associated adenomas and 1 adenoma) and 30 ampullary neoplasms (18 intestinal-type adenocarcinomas with associated adenomas, 6 pancreaticobiliary-type adenocarcinomas [4 with associated adenomas], and 6 adenomas). Subsequently, 25 test biopsy cases were stained with the identified panel of 5 antibodies and the predicted diagnoses were compared to follow-up information.

Results: There was almost complete concordance between the immunohistochemical staining patterns of PDA cells colonizing duodenal mucosa and the underlying infiltrating PDA (positive for CK7 and MUC1, negative for CK20, CDX2 and MUC2). In contrast, most duodenal and intestinal-type ampullary neoplasms were positive for CK7, CK20, CDX2 and MUC2, and negative for MUC1 (whether adenoma or invasive carcinoma). Using this information on 25 endoscopic biopsies from ampullary and duodenal lesions, it was possible to accurately predict colonization in 4/4 cases (3 underlying PDA and 1 pancreaticobiliary-type ampullary adenocarcinoma) and adenomas in 18/21 cases (2 were ambiguous and 1 was mistakenly called colonizing PDA). Thus, the five-antibody panel achieved a sensitivity of 93% and a specificity of 97% in distinguishing between the two diagnoses.

Conclusions: An immunohistochemical panel (CK7, CK20, CDX2, MUC1, MUC2) can be used to accurately predict whether adenomatous-appearing epithelium in endoscopic biopsies from the duodenum and ampulla represents a primary adenoma or colonization from an underlying PDA, providing invaluable information for subsequent treatment decisions.

601 Genomic Instability in Non-Dysplastic Intestinal Biopsies Distinguishes Crohn's Disease (CD) Neoplastic Progressors from Non-Progressors Using Illumina 300K SNP Array Analysis

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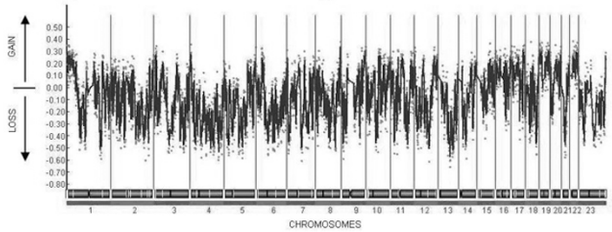
Background: Only a minority of CD patients will undergo neoplastic progression but no reliable means yet exist to identify this subset. Therefore, detection of curable neoplasia currently requires lifelong endoscopic surveillance for all CD patients. Because array-based genomic hybridization in the closely related disorder of ulcerative colitis has been shown to successfully distinguish neoplastic progressors (P's) from non-progressors (NP's) who do not progress while in long-term surveillance, using single nondysplastic biopsies, we now seek to examine its utility in CD.

Design: The Illumina 300K single nucleotide polymorphism (SNP) array was used to survey >300,000 sites throughout the human genome. Labeled DNA extracted from fresh frozen nondysplastic epithelium in CD biopsies and patient-matched constitutional DNA was used for hybridization in 5 CD-P's (7 total samples) versus 4 CD NP's (4 total samples). Sample locations included rectum, colon and ileum and were located a mean of 44 cm from the tumors. Global assessment of whole genome plots was performed by applying a moving average of 2Mb such that only changes spanning many SNP's were detected.

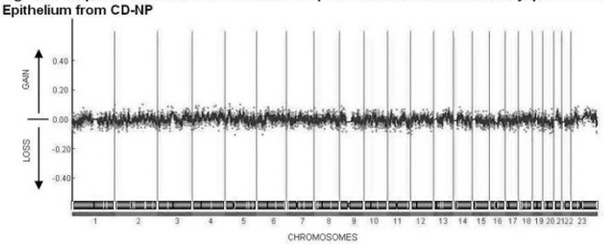
Results: Low to high variability genomic gains and losses were found in 4 of 7 CD-P samples derived from 3 of 5 CD-P patients (figure 1).

Figure 1. Representative Illumina 300K SNP Chip Whole Genome Plot of Nondysplastic Rectal Epithelium from CD-P with Descending Colon Cancer



None of the 4-NP's showed significant genomic alteration beyond baseline (figure 2).

Figure 2. Representative Illumina 300K SNP Chip Whole Genome Plot of Nondysplastic Ileal Epithelium from CD-NP



Conclusions: Pan-genomic alterations exist in CD-P's and are present even in nondysplastic tissue obtained far distant from neoplasms. Genomic alterations are not present in NP's and therefore show promise for future biomarker development to distinguish CD-P's from NP's.

602 Slouging Esophagitis: A Type of Contact Esophageal Injury in Debilitated Patients?

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Background: Sloughing esophagitis (SE) is characterized by a superficial mucosal necrosis that produces endoscopic white plaques or membranes. According to the only previous report of 4 cases, it occurs in older, debilitated patients who are on multiple medications, suggesting it is a contact injury. This study of 24 cases was undertaken to validate these findings.

Design: 24 patients with biopsy-proven sloughing squamous epithelium, negative for fungi, and with endoscopic white plaques or membranes, were compared with a control group of 34 patients with esophageal biopsies for any purpose other than Barrett's surveillance.

Results: SE patients had a median age of 53.5 years (controls 43.5 years). SE patients were significantly more likely than controls to be taking 5 or more prescription medications (71% vs. 32%), especially CNS depressants such as narcotics and benzodiazepines (62% vs. 32%), and medications known to be associated with esophageal injury (54% vs. 18%). The endoscopic membranes were most often in the distal (70%) and/or mid-esophagus (45%). 20% of cases involved the entire esophagus; 10% were located only in the proximal esophagus. There was no correlation between medication and site of SE. SE patients were significantly more likely to have clinical features of chronic debilitation, including being unable to work/on disability, on home oxygen therapy, residing in assisted living/nursing home, being bedridden, hospitalized > 1 month, malnourished, metastatic cancer, organ transplantation in preceding 6 months, and/or on immunosuppressive therapy. SE patients were more likely than controls to have died since the biopsy (25% vs. 3%), with a mean time to death of 120.5 days. SE patients were significantly more likely than controls to have a history of peptic ulcer disease (62% vs. 24%), but were no more likely to have dysmotility disorders, irritable bowel disease, or atherosclerotic vascular disease. SE patients were less likely than controls to have gastroesophageal reflux disease (GERD) (50% vs 74%).

Conclusions: SE patients are likely to be on multiple drugs, including CNS depressants and those which directly injure esophageal epithelium, and are likely to be debilitated. Interestingly, they are less likely than controls to have GERD. No specific cause for SE was identified, but the associations with drugs and clinical conditions that may lead to esophageal stasis and/or injury, and the lack of association with atherosclerotic diseases suggest that this is a direct contact injury, rather than an ischemic injury.

603 HER2 Positivity in Advanced Gastric Cancer: Variation in Histopathological Staining Compared to Breast Cancer

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Background: Accurate assessment of human epidermal growth factor receptor 2 (HER2) status is required to identify patients eligible for treatment with trastuzumab. In gastric cancer (GC), HER2-positivity rates of 6-35% have been reported, though this wide variation is likely due to small sample sets and differing methods of evaluation or scoring. A specific HER2-testing process was established for the Phase III ToGA trial (trastuzumab with chemotherapy in HER2-positive advanced GC), the largest prospective trial analysing HER2 status in GC.

Design: A validation study was completed to standardise IHC (HercepTest™) and FISH (pharmDx™) protocols, and to establish a scoring system specific for GC (Hofmann et al. Abstract 24; ASCO 2006 Gastrointestinal Cancers Symposium). Tumour samples were centrally tested by both IHC and FISH to identify patients eligible for enrolment in the ToGA trial.

Results: To date, 2083 tumour samples have been assessed (460 HER2 positive; 1623 HER2 negative), giving an overall HER2-positivity rate of 22.1%. The concordance between IHC and FISH results for 1876 patients was lower (87.3%) than what is typically observed in breast cancer (95%). This lower concordance was largely due to FISH+ cases that were IHC 2+. Of the 460 HER2-positive GC samples, ~8% were FISH+ and IHC 0, which is unusual compared to breast cancer. A higher level of heterogeneity in both HER2 protein expression and gene amplification compared to breast cancer was also observed. HER2 positivity differed significantly by histological subtype: 33.4% (349/1044) in intestinal, 5.5% (35/636) in diffuse and 19.6% (74/378) in mixed. HER2 positivity also varied according to the site of the tumour: 33.6% (37/110) for gastro-oesophageal junction tumours and 19.9% (222/1118) for gastric tumours.

Conclusions: Using a validated methodology and the large sample set from the ongoing ToGA trial, the HER2-positivity rate observed in advanced GC (~22%) is comparable to that in breast cancer. Variations in HER2 positivity were observed depending on tumour site and histological subtype. The role of these variations in HER2 positivity and the differences between FISH and IHC results with regards to trastuzumab response will be of great interest. The first efficacy data from ToGA are expected in 2009.

604 Thrombotic Microangiopathy of the Gastrointestinal Tract

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Background: Thrombotic microangiopathy (TMA) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and variable signs of organ dysfunction due to platelet aggregation in the microcirculation. It includes but is not limited to the syndromes of thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). TMA has been well characterized in the kidney and other organs. There are very few studies detailing the clinicopathologic features of TMA in the GI tract; it may be underappreciated in cases of ishcemic injury to the GI tract.

Design: We review the clinical and pathologic features of 7 patients with the diagnosis of TMA or probable TMA involving the GI tract.

Results: There were 4 male and 3 female patients, ranging in age from 18-61. Five presented with GI bleeding (2 upper, 3 lower), 1 with epigastric pain, and 1 with intractable watery diarrhea. The clinical diagnosis of TMA was not considered prior to histologic exam in any patient. All cases demonstrated acute and organizing fibrin thrombi in submucosal arteries, arterioles, or capillaries. One case had no significant mucosal injury, the other 6 had varying degrees of acute and chronic ischemic type injury. In 4 patients a definitive clinical diagnosis of TMA could be established: 2 of these patients had SLE with concurrent renal biopsies demonstrating TMA. One had undergone renal transplantation; the other had CMV infection. One patient, had undergone lung transplantation and then developed TTP. One patient, had undergone kidney transplantion secondary to HUS, and developed malignant hypertension. In 3 cases, a definitive diagnosis of TMA could not be established. One patient had SLE complicated by presumed lupus vasculitis, renal vein thrombosis, acute renal failure, and episodes of malignant HTN. Neither definitive vasculitis nor antiphospholipid syndrome was demonstrated. One patient with HIV/AIDS had CMV esophagitis, and had been treated for *C. difficile* infection. In these 2 patients, nonspecific thrombosis of submucosal vessels in an area of erosion could not be definitively distinguished from TMA as a cause of ischemic injury. One patient s/p orthotopic liver transplantion had numerous thrombosed submucosal vessels and rare CMV inclusions.

Conclusions: Ischemic changes in the GI tract may be due to TMA. The presence of fibrin thrombi is a subtle histologic clue to a clinically under-appreciated process which in some cases is systemic and has important treatment implications. TMA should be considered in the differential diagnosis of ischemic change in the GI tract.

605 Immunohistochemical Demonstration of Esophageal Dilated Intercellular Spaces (DIS) in Squamous Mucosa

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Background: Criteria for the histological diagnosis of esophageal acid-related diseases in squamous mucosa have been hampered by lack of sensitivity and specificity. The possibility that dilated intercellular spaces (DIS) might be a sensitive marker of gastro-esophageal reflux disease (GERD) and non-erosive reflux disease (NERD) has been suggested.

Design: To determine whether immunohistochemical stains might demonstrate DIS with greater ease than H and E sections, biopsies of squamous mucosa (two form the Z-line, two form 2cms above the Z-line, one from 8cm above the Z-line, and one from the most distal erosion if present), were examined in 50 patients with acid-related GI symptoms as part of a randomized control trial. All patients underwent endoscopy and biopsy from these sites both pre-therapy and after 6 months of therapy (esomeprazole 40mg od or 20mg od prn), and all biopsies were oriented on filter paper. Four immunostains (desmocollin2, desmoglein2, CD15 and b-catenin) were compared to standard HE sections and to each other. DIS were scored on a 0-3 grading system as 0, 1 or 2, 3-5 or >5 DIS/x63 hpf respectively. The highest score recorded was used in view of the potential focality of GERD.

Results: DIS were preferentially located in the suprabasal and peripapillary regions of the squamous mucosa. All immunostains identified slightly different areas of the squamous epithelium. b-catenin and desmoglein2 were best able to demonstrate DIS with ease and separate them from cytoplasmic vacuoles. They also showed the greatest difference in pre- and post-treatment biopsies regarding scoring of DIS, and correlated well with scores obtained with HE sections (all p values <.025).

Conclusions: b-catenin and desmoglein2 allowed the best demonstration of DIS as scored in both pre- and post-treatment biopsies of squamous mucosa in patients with acid-related disease. As b-catenin is more likely to be available in routine practice, and was less capricious, its use as a potential marker of DIS is suggested. This study also supports the evaluation of DIS as a marker in patients with acid-related diseases that can be used at the light microscopic level.

606 Prostate Specific Membrane Antigen (PSMA) Protein Expression in Colorectal Cancer (CRC)

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Background: PSMA, a transmembrane folate hydrolase, is expressed at low levels in normal prostate epithelium and is significantly overexpressed in prostate cancer (PCA) and the neovasculature of various non-PCAs. Endothelial PSMA expression has not been previously studied as a prognostic factor for CRC.

Design: Formalin-fixed paraffin-embedded tissue sections from 130 cases of CRC were immunostained by an automated method (Ventana Medical Systems Inc., Tucson, AZ) using predilute mouse anti-human PSMA (clone 3E6; cat# N1611, DAKO Carpinteria, CA and CD31 mouse anti-human CD31 (clone JC70A; cat# M0823, DAKO Carpinteria, CA). Both tumor cell (TC) and tumor vascular endothelial cell (TVE) immunoreactivity was semiquantitatively scored based on staining intensity and distribution and the results were correlated with standard morphologic and prognostic variables.

Results: PSMA immunoreactivity in TVE was absent or low in 36 (28%) cases and high in 94 (72%) cases. 115 (88%) cases had absent or low PSMA expression in TC and 15 (12%) had high TC expression. PSMA staining of CRC TC was focal and granular compared to the diffuse homogeneous staining of TC in control cases of high grade PCA. High TVE PSMA expression strongly correlated with gender 37/67 (55%) male vs 21 /63 *(*33%) female, p=0.009) and tumor grade 11/21 (52%) high grade vs. 47/109 *(*43%) low grade, p=0.046). Patients with strong TVE PSMA expression in their CRC had a shorter overall survival than patients with PSMA negative TVE (52 months vs 61 months, mean overall survival 58 months), but this difference did not reach statistical significance. There were no other correlations with clinicopathologic parameters assessed in this study.

Conclusions: This study confirms the presence of PSMA expression in the TVE of CRC and, for the first time, correlates this expression with clinical and disease outcome parameters. This study also identified the uncommon expression of PSMA in CRC TC. Given the potential clinical utility of anti-PSMA imaging for management of CRC and potential targeting of anti-PSMA pharmaceuticals to the CRC tumor vasculature, further study of PSMA expression in CRC appears warranted.

607 CDX-2, Cytokeratins 7 and 20 Immunoreactivity in Rectal Adenocarcinomas

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Background: Colonic mucosa is typically positive for cytokeratin 20 (CK20) and negative for CK7. This immunophenotype has been used to distinguish colonic adenocarcinoma from carcinomas arising from other organs. Some studies have reported frequent CK7 positivity in rectal adenocarcinomas. CDX-2 has been shown to be a specific and a sensitive marker for colonic carcinoma. However, there is limited data about its expression in rectal carcinoma. In this study, we investigated the diagnostic utility of CK7, CK20 and CDX-2 markers in a series of rectal adenocarcinomas.

Design: Fifty five specimens of rectal adenocarcinomas were retrieved from the archives of anatomic pathology. Thirty cases of pancreatic adenocarcinoma and 15 cases of cholangiocarcinoma were also included as a control group. All cases were stained for CK7 (Dako-M7018), CK20 (NovoCastra NCL-L-CK20) and CDX-2 (NovoCastra NCL-CDX2). For CK7 and CK20, cytoplasmic staining was considered positive, while for CDX-2 only nuclear staining as positive. Percentage of cells staining was recorded as follow: Negative (0-5%), weak (1+, >5-25%), moderate (2+, >25-50%) and strong (3+, >50%).

Results: CK7 was expressed in 12/55 (22%) of rectal carcinoma as follow: 4 weak and 8 with moderate-to-strong positivity. CK20 was positive in 48/55 (87%) rectal carcinoma: 4 weak, 13 moderate, and 31 cases with strong positivity. There were six negative cases (7%) for CK20, with a positive internal control. CDX2 showed moderate-strong positivity in all cases: 12/55 (22%) moderate and 43/55 (78%) strong positivity which was not related to tumor differentiation. Benign rectal mucosa was available in 37 cases and showed the following results: CK20+/CK7- in 25/37 (67%), CK20+/CK7+ in 8/37 (22%) and CK20-/CK7- in 4/37 (11%) cases. In pancreatic and cholangiocarcomas, 29/45 (64%) were CK7+/CK20+ and 16/45 (36%) were CK7+/CK20-. CDX-2 was weakly positive in 3/45 (7%) cases, all were pancreatic adenocarcinoma.

Conclusions: CK7 can be expressed in rectal adenocarcinoma, and should not be used as the sole basis for excluding a rectal primary. In addition, rectal carcinoma can also be CK7-/CK20-. Our study shows that CDX-2 is a sensitive and a specific marker for rectal origin of the adenocarcinoma, and can be helpful in cases with metastatic rectal carcinoma; especially those with CK7 positive or CK20-/CK7- cases. In contrast to colonic carcinoma, CDX-2 expression was not influenced by the grade (differentiation) of the rectal adenocarcinoma.

608 Non-Adenomatous Dysplasia in Barrett's Esophagus: A Clinical, Pathologic and Molecular Study

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Background: Rarely, dysplasia in Barrett's esophagus (BE) is composed of crypts lined by cuboidal-shaped cells that contain a centrally located nucleus, markedly increased N/C ratio, but without nuclear stratification (termed "non-adenomatous" dysplasia: NAD), the latter of which is the characteristic feature of traditional "adenomatous" dysplasia (AD). The aim of this discovery study was to evaluate the clinical and pathologic features, natural history, and flow cytometric abnormalities of BE patients with NAD enrolled in a high-risk BE prospective surveillance program.

Design: Eighteen BE patients with NAD (28 biopsies, 47 foci), identified over a 6 ½ year period (07/01-02/07) from our BE cohort that included 310 consecutive patients, were evaluated for a wide variety of clinical and pathologic features, including association with traditional AD and cancer, and their flow cytometric characteristics (increased 4N, aneuploidy). The data were compared to the other 275 (of 292) BE patients without NAD (controls) who also had flow cytometry analysis performed (17 patients did not have flow cytometry).

Results: In our high-risk cohort, the prevalence rate of NAD was 5.8% (1 female, 17 males, mean age: 66.7 yrs., mean length of BE: 3.9 cm). 10 patients (44%) had one focus of NAD, and 8 (56%) had multiple foci at different levels of the esophagus. 62% and 38% of NAD foci were associated with goblet or non-goblet epithelium, respectively. Most patients (89%) also had foci of AD (39% high grade) either previously (72%) and/or concurrently (89%). Of the NAD patients with follow-up, all (100%) developed further foci of NAD, 71% also developed further AD (mostly high grade), and 4 (22%) either had (n=1), or developed (n=3) cancer. Compared to controls (N=275), BE patients with NAD showed a significantly higher rate of flow abnormalities (38.5% vs. 10.5%, P=0.01) and association with cancer (22% vs. 4%, p=0.01), but no differences were noted with any of the other clinical or pathologic features evaluated.

Conclusions: Non-adenomatous dysplasia is a rare (prevalence 5.8%) variant of in-situ neoplasia in BE that, based on our data, should probably be considered a "high-risk" lesion for the purposes of patient management, due to its high rate of flow abnormalities and association with traditional high grade AD and cancer.

609 Inverted Hyperplastic Polyp of the Colon: A Hitherto Unrecognized Pitfall in the Diagnosis of Sessile Serrated Adenoma

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Background: Colonic hyperplastic polyp (HP) is essentially benign while sessile serrated adenoma (SSA) has been shown to be related to microsatellite unstable colorectal carcinoma. The diagnosis of SSA is based on architectural features that are subject to interobserver variability. We hereby report anecdotal observations of several features that may affect the diagnosis of SSA.

Design: The study group consists of 20 inverted serrated polyps (IVP) which were identified during the sign-out due to difficulty to assign a firm diagnosis of HP or SSA. Controls include 30 SSA and 30 size-matched HP without inverted growth pattern. The diagnosis of SSA was made on H&E slides which show at least 2 of 4 features (dilatation, branching, serration, and horizontality) of basal crypts. Each polyp was noted for its location (right or left colon, separated by splenic flexure) and the presence or absence of submucosal lipoma, and immunohistochemically stained with antibody to MUC6 (a gastric mucin).

Results: The 60 control SSA and size-matched HP were evenly distributed in size (3 to 17 mm). 25/30 (83%) SSA were located in the right colon and 29/30 (97%) HP were left-sided. 15/30 (50%) SSA and no (0/30) HP had an associated submucosal lipoma. MUC6 was expressed in the basal crypts of all (30/30) SSA and in no (0/30) HP. In the study group, all IVP had at least one of 2 potentially confusing patterns (1) "lobular" pattern of misplaced glands in the submucosa and delineated by muscularis mucosa; or (2) "prolapse" pattern of protruded basal crypts still attached to native muscularis mucosa. All "lobular" nodules had at least 3 features of basal crypts associated with SSA, mostly dilatation, serration, and horizontality. All "prolapse" mucosa had at least 2 aberrant basal features, frequently branching or dilatation. All 4 MUC6(+) IVP were right-sided, with underlying lipoma and were therefore assigned to SSA. The other 16 MUC6(-) IVP had at least 2 aberrant features only seen in the lobular or prolapse areas, but were assigned to HP based on left-side location, absence of lipoma and negative MUC6 staining.

Conclusions: IVP is a potential diagnostic mimic of SSA. The morphological diagnosis of SSA should be made on noninverted mucosa rather than on areas of inverted growth. Location in the right colon, presence of lipoma, and positive MUC6 immunostain all favor the diagnosis of SSA.

Features of Polyps

Polyp Type	Case No.	Colon Site (Rt/Lt)	Lipoma (+)	MUC6(+)
SSA	30	25/5	15	30
HP	30	1/29	0	0
IVP	20	4/16	4	4

610 Prognostic Significance of Lymphangiogenesis in Rectal Adenocarcinoma

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Background: Lymph node metastases is a major prognostic indicator for disease progression and crucial for therapeutic strategies in rectal adenocarcinoma. There is limited data evaluating the significance of lymphatic microvessel density (LMD) in rectal adenocarcinoma. In this study, we investigated D2-40, a lymphatic marker, as a

predictive marker for the risk of lymph node (LN) metastasis, local recurrence and its relation to other prognostic parameters in rectal adenocarcinoma patients.
Design: Surgical specimens from 65 patients with rectal adenocarcinoma treated with complete total mesorectal resection and node dissection were immunostained for D2-40 and CD31. Positively stained microvessels (MV) were counted in densely vascular/lymphatic foci (hot spots) at x400 field in each specimen (=0.17 mm2) by 2 pathologists. Results were correlated with clinicopathologic prognostic parameters.
Results: D2-40 identified peritumoral lymphatics in all cases and intratumoral lymphatics in 52/65 (80%) cases. CD31 MV counts showed significant correlation with depth of invasion and distant metastases (r=0.31, 0.34; P< 0.05). However, there was no correlation between CD31 MV and presence of angiolymphatic invasion, lymph node metastases and disease stage. D2-40 detected angiolymphatic invasion (LVI) in 27/65 (42%) cases, more than with CD31 (22/65, 34%) and H&E (20/65, 31%). LVI detected by D2-40 demonstrated significant correlation with LN metastases, stage of the disease and local recurrence (r=0.49,0.51,0.37, respectively, P< 0.05). Peritumoral D2-40 LMD demonstrated a significant correlation with size of the tumor, lymph node metastases, presence of angiolymphatic invasion and stage of the tumor (r= 0.41,0.32, 0.35, 0.30, respectively, P<0.05), while intratumoral LMD demonstrated significant correlation with tumor size and depth of invasion (r=0.46,0.31, P< 0.05). In our study, there was a significant correlation between status of the radial margin and local tumor recurrence (r=0.73,P<0.0001).
Conclusions: Immunostaining with D2-40 increases the frequency of detection of lymphatic invasion relative to H&E and the commonly used pan-endothelial marker, CD31. Our study showed that both angiogenesis and lymphangiogenesis play an important role in the progression of rectal adenocarcinoma. D2-40 LMD showed prognostic significance with positive correlation with angiolymphatic invasion, metastases to lymph nodes and tumor stage. D2-40, by staining lymphatic vessels and detecting lymphatic invasion in rectal adenocarcinoma, is a useful immunohistochemical marker for tumor progression.

611 Microarray Gene Expression Analysis of High Grade Dysplasia in Barrett Esophagus

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Background: The aim of this study was to screen for more precise molecular markers of dysplasia using laser capture microdissection (LCM) and gene microarray technology in biopsies from patients with Barrett esophagus (BE).
Design: Laser capture microdissection (LCM) was employed to capture high grade dysplastic (HGD) epithelium and non-dysplastic (ND) intestinal metaplasia from 11 paired biopsy samples (each from the same patient). mRNA was reverse transcribed and applied onto Affymetrix cDNA microarray chips customized for mRNA from paraffinized, formalin-fixed tissue. Differentially expressed genes were identified by applying a supervised analysis using the significance analysis of microarrays (SAM) method.
Results: Genes expressing at least a two fold change in the HGD versus ND groups and a false detection rate (FDR)<20% were used for annotation and classification into functional groups. When comparing between HGD and ND epithelium two fold or greater increased expression was observed in 168 genes including several genes known to be increased in HGD as well as many novel candidates including; lipocalin 2, S-100A9, MMP-12, secernin 1 and topoisomerase II. A two fold or greater decreased expression was found in 72 genes including mucins 3A, 5A and B, trefoil factors 1 and 2, meprin A, alanyl-aminopeptidase and others. A subset of these genes was subsequently validated by real-time PCR and immunohistochemistry on these same samples and on additional biopsies from new patients.
Conclusions: LCM of archival paraffin embedded specimens has enabled the identification of several genes whose expression is altered in HGD. This study has confirmed the presence of genes previously implicated in the pathogenesis of Barrett's associated dysplasia and highlighted novel candidates for further investigation.

612 Tumor Expression of Integrin-Linked Kinase (ILK) Correlates with the Expression of the E-Cadherin Repressor Snail: An Immunohistochemical Study in Ductal Pancreatic Adenocarcinoma

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Background: Pancreatic adenocarcinoma is a lethal disease with a 5-year survival rate of 4%. Currently, the only opportunity for improved survival is complete surgical resection for cases of localized disease. Integrin-linked kinase (*ILK*), which is a key molecule involved in mediating extracellular events and intracellular survival pathways, as well as activating the downstream transcription factors AP-1 and NFk B, has been demonstarted to be expressed in ductal pancreatic adenocarcinoma and be linked to tumor prognosis. Previous studies in colon cancer have shown expression of ILK dependent with the tumorgrade. The present study was thus initiated to examine ILK expression dependent on the tumor grade and neoplastic precursor lesions, namely pancreatic intraepithelial neoplasia (*PanIN*).
Design: Resection specimen of 24 randomly selected patients, who underwent a pyloric preserving pancreatoduodenectomy at our institution in 2006 for ductal pancreatic adenocarcinoma, where utilized for this study. Pancreatic tissue was stained for ILK, E-cadherin and Snail in a standard fashion. Slides were reviewed by two pathologists and graded accordingly. Staining positivity was scored, as either focal (<10% of tumor cells positive), moderate (10-50%) or extensive (> 50%). The Spearman rank-order correlation coefficient (P) was used to assess bivariate association.
Results: In 22 of 24 cases ILK expression within the ductal pancreatic adenocarcinoma showed extensive positivity, while 2 cases did not demonstrate any ILK staining. PanIN grade 1 (n=16) and 2 (n=11) lesions did not exhibit any ILK staining activity, whereas PanIN-3 (n=19) lesions demonstrated focal positivity for ILK. E-cadherin showed a reciprocal staining pattern to ILK in 21of 24 cases, with low (< 10%) expression of the marker in poorly differentiated adenocarcinoma. 15 of 19 PanIn-3 lesions, however, expressed high E-cadherin staining. Snail expression (n=22) correlated with ILK expression in ductal pancreatic adenocarcinoma (P=0.08), but no Snail staining activity was detected in PanIN lesions.
Conclusions: Besides demonstrating a tumour grade dependent expression of ILK within pancreatic adenocarcinoma, this study also demonstrated an increase of ILK within high grade PanIN lesions. The increase in expression of the E-cadherin repressor Snail, may point towards an ILK-mediated induction, as previously shown.

613 Clinical and Histologic Predictors of Outcome in Patients with Colonic Crohn's Disease

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Background: The clinical and pathologic features of Crohn's disease (CD) restricted to the colon (CC) are variable, and ranges from appearance similar to ulcerative colitis (UC-like) at one end of the spectrum to those that resemble classical CD of the ileum at the other. Several previous studies have shown that a subset of patients with CC have a good outcome after either subtotal or total colectomy with ileal pouch-anal anastomosis (IPAA). The aim of this study was to evaluate clinical and pathologic parameters that may help predict a successful outcome of CC patients after their initial surgical procedure.
Design: A wide variety of clinical and pathologic features were evaluated in colon resection specimens from 44 patients with CD limited to the colon at the time of initial presentation. The histologic parameters were separated into major (such as granulomas, transmural lymphoid aggregates, fissuring ulcers, fistulas, skip lesions) and minor categories. The patients were identified from over three-hundred cases of CD at two major hospitals. The pathologic features in the colectomy specimens were correlated with outcome, categorized as either favorable (no complications) or unfavorable (≥1 of the following complications: recurrent disease, pouchitis, fistulas, small intestinal involvement). The mean follow-up was 46 months.
Results: Clinically, a younger age at diagnosis (30 vs 43 years) was significantly associated with an unfavorable outcome by multivariate analysis (P=0.03). Pathologically, skip lesions in the colon were always associated with a poor outcome (P=0.06) and the presence of active disease at a resection margin showed a trend towards an association with poor outcome (P=0.07). Patients with either none or only one major CD feature showed a poor outcome in 42% of cases, compared to 67% and 100% of cases in patients with two or three CD-like features, respectively. In a subset of patients with no major CD features (UC-like) and clinically confirmed CD (n=9), only one developed ileal disease upon follow-up.
Conclusions: Patients older than 43 at the time of diagnosis with either no (UC-like) or only one major CD-like histologic feature in their colectomy specimen have a higher likelihood of having a successful outcome post colectomy and IPAA.

614 Immunohistochemistry (IHC) as First-Line Screening Tool for Detecting Colorectal Cancer (CRC) Patients at Risk for Lynch Syndrome: A 2-Antibody Panel Is as Predictive as a 4-Antibody Panel

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Background: Immunohistochemical staining for DNA mismatch repair (MMR) proteins has been shown to be a simple and useful tool in screening CRC patients at risk for Lynch Syndrome. Currently, in both research and clinical settings, a 4-antibody panel that covers the 4 most commonly affected genes (*MLH1, MSH2, MSH6* and *PMS2*) is being used. Based on the fact that MMR proteins form heterodimers (MLH1 with PMS2, MSH2 with MSH6) and the function of individual proteins depends on the stability of the dimers, we hypothesized that a 2-antibody panel, using one from each dimer, would be sufficient to detect abnormalities in all 4 proteins.
Design: MLH1, MSH2, MSH6 and PMS2 were tested by IHC on CRCs in 2 patient cohorts: 1) a prospectively accrued consecutive series of 190 patients who were judged to carry a higher-than-average risk for hereditary colorectal cancer syndromes based on age and/or clinical history; and 2) a retrospective series of 42 patients who were age 40 or younger.
Results: Stains were regarded as negative (protein lost) when there was no nuclear labeling in tumor cells (with positive internal control). As summarized in the Table, the most common abnormality was concurrent loss of MLH1 and PMS2 (seen in 17% of the cases), followed by concurrent loss of MSH2 and MSH6 (6%). Notably, all MLH1- and MSH2-abnormal cases were also abnormal for PMS2 and MSH6 respectively, whereas 9/50 (18%) PMS2- and 6/20 (30%) MSH6-abnormal cases showed loss of staining for PMS2 or MSH6 alone (with normal MLH1 and MSH2).

Table. MMR IHC results.

IHC patterns	Prospective series	Retrospective series	Total
Loss of MLH1 alone	0	0	0
Loss of PMS2 alone	9	0	9 (4%)
Concurrent loss of MLH1 and PMS2	38	3	41 (17%)
Loss of MSH2 alone	0	0	0
Loss of MSH6 alone	5	1	6 (3%)
Concurrent loss of MSH2 and MSH6	10	4	14 (6%)
All 4 proteins present	128	34	162 (70%)
Total	190	42	232

Conclusions: The observed staining patterns are in keeping with the biochemical properties of the proteins, i.e., MLH1 and MSH2 are obligatory partners in their respective heterodimers whereas PMS2 and MSH6 are not. Thus, as a screening tool to detect MMR deficiency, a 2 antibody panel (PMS2 and MSH6) is as efficient as

the current 4-antibody panel and would reduce the cost by half. Such a cost-effective approach carries significant implications as IHC is being widely adopted as one of the first-line screening tools for Lynch Syndrome.

615 Clinicopathologic Characteristics of Intraglandular Necrotic Debris in Colonic Biopsy and Surgical Specimens

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Background: Intraglandular necrotic debris (IND) in gastric biopsy specimens has been reported as a diagnostic clue to non-invasive high-grade dysplasia or invasive carcinoma. It is defined as an eosinophilic material with necrotic epithelial fragments within the lumen of a dilated atypical gland. We investigated the clinicopathologic characteristics of IND in both biopsy and surgical specimens.

Design: We reviewed a total of 153 colonic biopsy, polypectomy, and endoscopic mucosal resection (EMR) materials (397 lesions) and 29 surgical specimens. These specimens were given the following pathologic diagnoses: 1) non-neoplastic lesions, 2) adenomas (tubular, tubulovillous, villous, and serrated), and 3) adenocarcinomas. Adenomas were further graded into mild, moderate, or severe degree. The number of IND per one section was also divided into three groups: 1) none, 2) 1 to 5, 3) above 5. The size of IND was also divided into three groups (small, medium, or large). We also evaluated the correlation between IND and age, sex, location in the colon, size of the tumor, and histological aubtypes of adenocarcinoma in biopsy, polypectomy, EMR, and surgical specimens.

Results: IND was found in only 4% of non-neoplastic lesions, and most cases were observed in neoplastic lesions, especially in cases of adenocarcinoma (11% of adenoma and 51% of adenocarcinomas). The number of IND, especially above 5 per section, was seen only in casses of adenocarcinoma. Regarding the adenoma, IND was found in tubular and tubulovillous adenomas, and most of them showed moderate to severe dysplasia. IND was not observed in villous and serrated adenomas. In cases of adenocarcinoma, moderate differentiated adenocarcinoma showed the highest incidence of IND. The incidence of IND was higher in polypectomy and EMR specimens than that in biopsy. In surgical specimens, all cases showed the presence of IND.

Conclusions: IND was frequently found in colonic neoplastic lesions, especially in cases of adenocarcinoma. The frequency of IND in cases of adenocarcinoma of the colon is not so high compared to thath in the stomach. However, if present above 5 per one section, it is necessary to keep in mind the possibility of adenocarcinoma.

616 Difficulty in Predicting Behavior of Small Bowel Carcinoid Tumors: Neither ki-67 Index nor Coronin 1C Staining Aids in Forecasting Outcome

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Background: It is difficult to predict the behavior of carcinoid tumors of the small bowel. Some studies indicate that proliferation index and presence of angioinvasion may aid in distinguishing benign and malignant carcinoids, but results have been mixed. Coronins are a family of actin-binding proteins that play a critical role in cell migration, cytokinesis, and phagocytosis. Prior studies suggest that coronin 1C shows predictive value for progression in some human cancers.

Design: Small bowel carcinoids were studied from 35 patients (16 men and 19 women, mean age 60.4 years, range 20-98). Staging information was recorded, including tumor size, extent of invasion, margin status, lymph node status, presence of satellite nodules and distant metastasis. Clinical follow-up was also obtained (mean 39 months, range 1-122 months). Tumor sections from each case were stained for chromogranin, synaptophysin, ki-67 and coronin 1C. Cell staining patterns were recorded and a 1000 cell count was used to determine ki-67 proliferation index. Statistical analysis was performed using SPSS software.

Results: The 35 carcinoid tumors had a mean size of 2.1 cm (range 0.4-10). Surgical resection was complete in 77%; 83% showed muscularis propria involvement, 46% involved the mesentery, 63% showed perineural invasion, 57% showed angioinvasion, 20% showed satellite nodules and 69% had positive lymph nodes. Distant metastasis was observed in 40%, mostly to the liver (10 of 12 cases). Disease recurrence was noted in 46% and death from disease in 23%. The only predictors of disease recurrence or death from disease were incomplete resection ($p=0.003$), mesentery involvement ($p=0.013$) and liver metastasis ($p=0.001$). Perineural invasion was associated with satellite nodules, mesentery involvement and liver metastasis ($p<0.05$). Tumor location, tumor size, muscularis propria involvement, lymph node status, angioinvasion, ki-67 index and coronin 1C staining were all non-predictive of recurrence, metastasis, and death from disease. Ki-67 indices ranged from 0-17.5 (mean 1.67) and a cutoff value for diagnosis was not evident.

Conclusions: For small bowel carcinoid tumors, completeness of primary resection remains the most important modifiable predictor of recurrence, metastasis and death from disease. Neither ki-67 index nor coronin 1C staining is useful in stratifying carcinoid tumors of the small bowel.

617 Lewis B (LeB) Antigen Expression in Colorectal Carcinoma – A Tissue Microarray (TMA) Study

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Background: Restricted expression of cell surface antigens in neoplasms rise the hypothesis that they can become potential targets for antibody-based immunotherapy of cancer. Monoclonal antibody (mAb) 58-1066 is regarded a potential therapeutic reagent to the Lewis B (LeB) antigen. This study was undertaken to determine the LeB antigen expression profile in a subset of colorectal carcinomas as well as in non-neoplastic mucosal counterpart, further assessing its usefulness as a potential target for immunotherapy.

Design: Paraffin blocks from 139 colectomy specimens were retrieved from Dept of Pathology of Hospital das Clinicas, University of Sao Paulo, Brazil. All cases were reviewed and normal as well primary tumor areas selected. Four TMA blocks were constructed with Beecher™ Manual Tissue Microarrayer, consisting of 126 normal and 130 primary tumor samples. Histological sections were immunhohistochemically analyzed with anti-LeB mAb 58-1066 employing a short-polymer-peroxidase kit, Novolink™, Novocastra, England. Extent as well as intensity and pattern (cytoplasmic, membranous) of immunostaining were analyzed semi-quantitatively.

Results: Mebranous staining was seen in 13.5% and 65.4% of non-neoplastic and neoplastic samples, respectively, ranging from 10% to 30% of cells in the normal and 10% to 100% in carcinoma group (means:1.59% vs 28.31%, $P<0.001$). Membranous intensities in mucosa were significantly lower ((0): 86.5%, (1): 7.1%, (2): 6.3%) when compared to tumoral samples ((0): 34.6%, (1): 13.8%, (2): 51.5%), $P<0.001$. Even cytoplasmic positivity was significantly higher in carcinomas when compared to mucosa (means: 16.98% vs 42.46%, $P<0.001$), as well the corresponding intensity distribution in normal mucosa (0): 39.7%, (1): 17.5%, (2): 42.9% versus carcinoma group (0):14.6%. (1): 22,3%, (2): 63.1% ($P<0.001$).

Conclusions: Although not exclusively restricted to colorectal carcinoma, the present study shows that LeB expression occurrs more frequently in cytoplasmic and membranous compartments of colorectal carcinoma cells compared to non-neoplastic colonic mucosa. This prevalent expression in carcinoma suggests that the LeB antigen may be a potentially useful target for antibody-based immunotherapeutic interventions.

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618 Glucose Transporter-1 (GLUT1) Expression & PET-CT-SUVmax in Esophageal Carcinomas

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Background: The prognosis of esophageal carcinoma (EC) correlates with the maximal standardized glucose uptake value (SUVmax) as measured by positron emission tomography (PET). Other prognostic variables include selected tumor-related proteins involved in cell cycle regulation, angiogenesis, and cellular proliferation. We hypothesized that expression of those moieties in EC might correlate with SUVmax values.

Design: The records and pathologic specimens from 67 EC cases that were treated with surgery alone (35 adenocarcinomas & 4 squamous cell carcinomas,group I) or surgery after neoadjuvant chemoirradation (24 adenocarcinomas & 4 squamous cell carcinoma, group II) were reviewed. Demographic data, tumor stage, histologic features, and SUVmax values were recorded. A tissue microarray was constructed for immunohistochemical studies that evaluated Ki-67, p53, VEGF, EGFR, cyclin-D1, and GLUT1. Statistical analysis of immunostaining results was performed using linear regression and Student's t-test.

Results: The median value of SUVmax was 4.8 (range 0-12.6) for group I ECs and 3.5 (0-6.4) for group II tumors. There were no differences in tumor type (adenocarcinoma vs. squamous carcinoma), tumor grade, level of necrosis or inflammation, or angiolymphatic invasion between groups. Group I lesions had more p53 immunoreactivity ($p< 0.04$) and a higher Ki-67 index ($p<0.001$) than did group II ECs. SUVmax correlated with tumor size and pathologic stage, but not with p53 status or expression of other proteinaceous markers. However, the SUVmax of EC did parallel the expression of GLUT1 ($p<0.03$) in both groups I and II.

Conclusions: GLUT1 immunoreactivity demonstrates a definable linear relationship with SUVmax values in ECs, as seen with PET. This finding may assist clinicians in choosing imaging studies after the surgical treatment of such tumors. PET-SUVmax measurements do not appear to correlate with other proteinaceous markers of tumor biology.

619 Patterns of Colloid Response in Residual Rectal Carcinoma Following Preoperative Radiotherapy. Association with Long-Term Survival

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Background: Preoperative radiotherapy (RT) improves outcome in patients with locally advanced rectal carcinoma and modifies the morphology of the tumour. Complete pathological response and down staging were the most powerful morphological outcome predictors, but the impact of other histological changes, especially colloid response, is not well define. The aim of this study was to identify patterns of morphologic alteration having a significant impact on prognosis.

Design: A series of 334 consecutive patients with T0-4_N0-2 rectal carcinomas preoperatively treated with RT and surgically resected between 1988-2007 were included. Pathological response to RT was determined using the MSKCC score (complete, near complete >96% and partial response) and stage was determined by the deepest residual viable tumor according AJCC staging system. Colloid pattern of response was defined by the presence of extracellular mucin pools with or without tumor cells. Positive lymph nodes, defined as having focus of viable tumor cells, and perineural (PNI) and lymphovascular invasion (LVI) were also recorded. Morphological findings were related with overall and relapse-free survival (OS and RFS) by univariate (Kaplan-Meier and Cox) and multivariate (Cox) analyses.

Results: Thirty nine of the 334 patients (12.4%) had a complete pathological response (pT0), and 54 (16.7%) had a near-complete response. Down staging was achieved in 136 patients (44.3%). Colloid response was found in 129 samples (40.8%). LVI and PNI invasion were seen in 64 (19.8%) and 66 (20.4%) of the patients. OS and RFS for the

entire series were 80.73% and 70.42% at 5 years, and 76.43% and 68.3% at 10 years, with a mean of 173 and 156 months respectively. There were significant differences (p<0,001) in OS and RFS in univariate analysis for all the factors except for colloid response (p=0.017 for RFS and 0.181 for OS). In Cox multivariate analysis the three factors retain the differences for RFS and OS. The presence of colloid pattern in complete response defined a small group of patients with less OS and RFS (78.57% and 100% v. 59.94% and 100%). There was no relationship between LVI and PNI invasion and local or distance relapse.

Conclusions: Complete or near complete response, LVI and PNI had a major impact on OS and DFS in rectal carcinomas treated with RT. Colloid pattern of complete response define a small group of patients with worse prognosis than non-colloid complete response.

620 Appendiceal Mixed Carcinoid-Adenocarcinomas Share Better Prognosis Associated with Goblet Cell Carcinoid Tumors

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Background: Appendiceal goblet cell carcinoid tumors (GCCT) have been reported to have a better prognosis whereas poorly-differentiated appendiceal carcinomas (PDAC), including those with mucinous or signet ring cell differentiation, usually confer a poor prognosis. However, the prognosis of appendiceal tumors that consist of GCCT with a component of adenocarcinoma (mixed carcinoid-adenocarcinomas, MCA) is unclear. In this study, we compared clinicopathologic features and prognosis of patients with GCCT, MCA and PDAC.

Design: A search of the surgical pathology database of the department of Pathology at M. D. Anderson Cancer Center from 1999 to 2007 yielded a total of 142 cases of appendiceal GCCT, MCA and PDAC. Clinicopathologic features were reviewed. Patients' follow-up information was obtained by reviewing medical records and the U.S. Social Security Index. Statistical analyses were performed using SPSS software (version 12.0; SPSS, Chicago, IL) and survival was evaluated by Kaplan-Meier analysis.

Results: 24 patients had GCCT, 26 MCA and 92 PDAC. Patients with MCA had a higher stage at presentation compared to those patients with GCCT, but a lower stage than those patients with PDAC. Among the 135 patients whose staging information was available, stage I-II, III and IV disease was present in 20 (87%), 3 (13%) and 0 (0%) in patients with GCCT; 13 (59%), 2 (9%) and 7 (32%) in patients with MCA; and 10 (11%), 13 (14%) and 67 (75%) in patients with PDAC, respectively (p=0.001). Mean overall survival was 66.3 ±18.1 months for patients with GCCT, 60.7 ±15.0 months for patients with MCA and 35.9 ±5.9 months for patients with PDAC (by log-rank method, p=0.007).

Conclusions: Patients with appendiceal MCA had a lower stage and better prognosis, similar to patients with GCCT. In contrast, patients with PDAC had advanced disease at presentation and poor prognosis.

621 Overexpression of COX-2 Predicts Poor Response to Neoadjuvant Chemoradiotherapy in Locally Advanced Rectal Adenocarcinoma

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Background: Preoperative chemoradiation for rectal adenocarcinoma reduces tumor size, increases the probability of a sphincter-saving procedure and improves local control. Some studies have also shown that complete pathologic response is a predictive factor for patient survival. However, there is a limited data about the utility of immunohistochemical markers as predictors for tumor response and downstaging in these patients. The objective of this study is to investigate the prognostic utility of MIB-1, p53, cyclin D1, Her2/neu, EGFR and COX-2 immunohistochemical markers in locally advanced rectal adenocarcinoma, and their relationship with clinicopathological response to neoadjuvant chemoradiotherapy.

Design: We identified 45 pretreatment biopsies from patients with locally advanced rectal adenocarcinoma who were treated with neoadjuvant chemoradiation. All patients underwent complete total mesorectal resection. Complete pathologic response was defined as absence of invasive carcinoma at resection, partial response as the presence of only few residual foci of adenocarcinoma located in mucosa, while no/poor pathologic response was defined as having invasive carcinoma involving at least the submucosa. Cases were immunostained for MIB-1, p53, cyclin D1, Her2/neu, EGFR and COX-2.

Results: Complete pathologic response was seen in 7/45 (16%), partial response in 8/45 (17%), and no/poor response in 30/45 (67%) patients. None of the clinical or pathologic variables in the pretreatment biopsy was associated with complete pathologic response. Overexpression of COX-2 was found in 30/45 (67%) cases and demonstrated significant correlation with lymphovascular invasion, depth of invasion, tumor stage, recurrence, and no/poor pathologic response (r=0.47, 0.4, 0.35, 0.32, 0.49, respectively; P< 0.05). EGFR was positive in 26/45 (58%) cases and was correlated with distant metastases and local recurrence (r=0.33, 0.41, P< 0.05). Other markers such as MIB-1, p53, Her2/neu, and EGFR showed no significant correlation with pathologic response or other pathologic parameters.

Conclusions: Our findings indicate that overexpression of COX-2 in the pretreatment biopsy correlates with poor response to neoadjuvant chemotherapy in locally advanced rectal adenocarcinoma patients. EGFR also showed prognostic significance by correlating with local recurrence and distant metastases.

622 High Grade Transformation of Differentiated Neuroendocrine Neoplasms (NENs) of the Enteropancreatic System – A Unique Entity Distinct from *De Novo* High Grade Neuroendocrine Carcinoma (HGNECa) in Pathogenesis and Clinical Behavior

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Background: The grading criteria for enteropancreatic NENs are controversial, but in general a high mitotic rate defines the aggressive group of HG NENs. Often designated HGNECa, these tumors exhibit poorly differentiated (PD-) histology, HG cytology, and poor outcome, and they are regarded as pathogenetically distinct from the family of differentiated NENs. However, in addition to these *de novo* PD-HGNECas, we propose that there exists a separate type of HG NEN that displays features of both a differentiated NEN and HG morphology.

Design: We reviewed the pathology and clinical records of the relevant cases. HG NENs were defined using the WHO classification of lung tumors (>10 mitoses/10 HPF). The features were compared with those of lower grade differentiated NENs and cases of *de novo* PD-HGNECa.

Results: Nine cases (7 pancreatic and 2 ileal) satisfied the entry criteria, and all presented with metastases (2 local, 7 distant). Five (56%) presented with carcinoid or related syndromes. All tumors exhibited foci of HG features, but had >50% component of differentiated NET morphology with lower grade cytology, and all were positive for synaptophysin or chromogranin. Serum chromogranin was elevated in all tested (6/6), and carcinoma antigens were negative (0/6). Since the majority were pancreatic primaries, we compared their features with differentiated endocrine neoplasms and *de novo* PD-HGNECas of the pancreas (Table).

	Age	Mean Size	Mitoses/ 50HPF	Ki67	Metastases	Mean FU (Mos)	NED/AWD/ DOD%
D-NEN (n=183)	56	4.7cm	0-36	1-10%	28%	44	63/10/20
HG-NEN (n=7)	51	6.3cm	>50	30-60%	100%	21	14/86/0
PD-HGNECa (n=8)	62	5.0cm	>50	50-95%	100%	14	25/unk/50

NED=no evidence of disease; AWD=alive with disease; DOD=died of disease

One with an ileal primary died in 9 mos, and all other 8 were alive with a mean follow up of 21 mos.

Conclusions: High grade transformation can uncommonly occur in differentiated NENs. Such tumors behave more aggressively than their low grade counterparts, but are not as aggressive as *de novo* PD-HGNECas. The association with a differentiated component suggests that some HG-NENs arise via a different pathway than most HGNECas, which are more commonly associated with an adenocarcinomas of their primary organs.

623 Glandular Carcinoids of the Ampulla with Psammoma Bodies (So-Called Ampullary Somatostatinomas): Analysis of 12 Cases of an Under-Recognized Entity

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Background: Carcinoids occurring in different parts of the GI tract have different clinicopathologic characteristics. A subset that occurs specifically in the ampulla has distinctive properties, which are under-recognized, largely because this entity has mostly been reported as a part of larger series on duodenal carcinoids or as individual case reports.

Design: Clinical and pathologic findings of 12 ampullary carcinoids with distinctive features that have been variably designated as glandular duodenal carcinoid or ampullary somatostatinoma, were analyzed.

Results: There were 9 females and 3 males with a mean age of 55 (28-85). The patients presented with gastric/esophageal ulcers (5), jaundice (3), GI bleeding (2), and 2 were detected incidentally. 3 were known to have neurofibromatosis (NF) (25%). No evidence of somatostatinoma syndrome such as diabetes or gallbladder/biliary disorders was identified. Mean size was 2 cm (1-4.5). The tumors tended to be well demarcated, round, forming a mucosal-covered nodule in the ampulla. In addition to the classical features of low-grade neuroendocrine neoplasms, all cases also displayed: 1) Lumen/tubule formation 2) Focal intraluminal mucin 3) Psammomatous calcifications. 1 case was originally diagnosed as adenocarcinoma, 1 as adenocarcinoid, and 1 as neuroendocrine carcinoma. 6/12 had LN mets. No distant mets were noted at the time of diagnosis. All expressed chromogranin and synaptophysin (diffuse/strong) and 10/12 expressed somatostatin. At the median F/U of 24 mos (1 to 96), 6 were alive with disease-free. One died post-operatively. No F/U was available on the remaining 5.

Conclusions: Low-grade neuroendocrine neoplasms of the ampulla with glandular pattern and psammoma bodies are a distinct subset of carcinoids seen predominantly in females, may be associated with NF, and can be mistaken for carcinomas due to tubule formation and intraluminal mucin. Although the tumors express somatostatin, they are not associated with the stigmata of somatostatin secretion, and therefore the term glandular psammomatous carcinoid of the ampulla is preferable to somatostatinoma. That many patients present with upper GI ulcers also speak against somatostatin activity. At the time of diagnosis, metastases to local LNs are seen in 50%, but liver mets are rare.

624 Alpha-Methylacyl-CoA Racemase (AMACR) Is Upregulated in Gastric Cancer: A Study of 220 Cases

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Background: AMACR (p504S) is a mitochondrial and peroxisomal enzyme involved in beta-oxidation of dietary branched-chain fatty acids and fatty acid derivatives. AMACR has recently been shown to be a biomarker that is expressed in a number of neoplasms,

such as prostate and colon cancer. Increased quantities of AMACR in cancer cells may serve as an enzymatic target for imaging or therapy in the new field of molecular radiology. AMACR expression in gastric neoplasms has not been fully investigated. The aim of this study was to evaluate AMACR expression in gastric neoplasms in a large cohort of patients.
Design: Tissue microarrays were designed with triplicate 1mm punch samples from 220 archived cases of gastric cancer. An immunohistochemical analysis of AMACR(1:100 anti-racemase; DakoCytomation, Carpinteria, CA) was performed in all cases. AMACR expression appears as a distinctive intracytoplamstic coarse granularity. The immunopositivity of each punch sample was graded on a scale from 0 to 3; 0 as no expression, 1 as weak, 2 as intermediate, and 3 as strong expression. The highest score of the three punch samples was recorded for each case. Non-neoplastic gastric tissues from various sites (e.g., the antrum, body, fundus, and pylorus) were used as controls.
Results: Overall, AMACR expression was found in 141 cases, 44 of which had weak expression, 47 intermediate, and 50 strong. Both intestinal and signet ring cell cancer overexpressed AMACR; intestinal cancer had significantly stronger expression than did signet ring cell cancer ($p<0.05$). Non-neoplastic gastric mucosa did not express AMACR.
Conclusions: The results of our study demonstrate that AMACR is upregulated in gastric cancer, suggesting that it may serve as an enzymatic objective for the new imaging and targeted therapy techniques; thus, its role as a potential target for therapeutic intervention should be explored further.

625 Characteristics of Epstein-Barr Virus–Associated Gastric Cancer: 20-Year Experience in a Single Medical Center

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Background: Studies have shown that Epstein-Barr virus (EBV) is associated with gastric cancer. However, the findings regarding the distribution of EBV infected cells (in normal gastric epithelium vs. intestinal metaplastic cells vs. in neoplastic cells) and characteristics of EBV-associated gastric cancer have been inconsistent. Lymph node positive EBV-associated cancer has not been systematically studied. The aims of this study were to evaluate EBV-associated gastric cancer, to assess the distribution of EBV infected cells including all positive lymph nodes, and to define characteristics of EBV-associated gastric cancer.
Design: We examined primary gastric cancer treated at M. D. Anderson Cancer Center between 1987 and 2006. Our study included only patients who underwent surgical resection with no preoperative treatment. Formalin-fixed paraffin-embedded tissue from these resection specimens were assessed for EBV by in situ hybridization (EBer kit, Ventana, Tucson, AZ), the gold standard for EBV detection in tissue. We analyzed EBV status along with clinicopathologic parameters that included age, and sex, as well as the location, size, histology, and pathologic stage of the tumor.
Results: Among 200 patients, 11 had intranuclear EBV. While all EBV-associated cases displayed EBV only in tumor cells, no detectable EBV was observed in normal gastric mucosa, intestinal metaplasia or stromal cells. Seven patients with EBV-associated gastric cancer had regional lymph node metastasis. Of note, positive EBV-infected tumor cells were observed in all lymph nodes with metastasis. The epidemiologic data of EBV-associated gastric cancer showed all 11 patients were male, ranging in age from 54 to 78 years old (mean, 60 years; median, 62.1 years), while gastric cancer not associated with EBV occurred in patients 21 to 93 years old (mean, 67 years; median, 66.4 years). No statistically significant association was found for other clinicopathologic variables analyzed.
Conclusions: EBV-infected cells are exclusively cancer cells. The presence of EBV-infected tumor cells in all lymph nodes with metastasis indicate that EBV is replicated along with immortal tumor cells. EBV-associated gastric cancer, a predominantly male phenomenon and more frequently seen in younger population, may be associated with certain life styles of the infected patients.

626 Non-Gastrointestinal Pathologists Are Highly Reproducible in Their Diagnosis of Serrated Polyps of the Colorectum Following Tutorial Instruction

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Background: Serrated polyps of the colorectum form a morphologically similar, yet molecularly diverse group of lesions which includes hyperplastic polyps, traditional serrated adenomas (TSA), and sessile serrated adenomas (SSA). The correct identification of these polyps is believed to be essential for appropriate colorectal cancer screening. Previous studies have shown variable reproducibility in the distinction of these serrated polyps by groups of gastrointestinal (GI) and non-GI pathologists. In this study, we aim to show that a group of non-gastrointestinal pathologists can achieve significant reproducibility following instruction in an educational program.
Design: A Powerpoint™ tutorial was created by the investigators (MT and JG) that contained written criteria and representative digital images of serrated polyps, based on the criteria published by Torlakovic et al. Following review of the tutorial, the participants (MBB, LCC, ALF, Hecht, Hunt, and MVR) were given 20 glass slides that represented well-oriented examples of the following entities: goblet cell hyperplastic polyp (GCHP), microvesicular hyperplastic polyp (MVHP), TSA, SSA and adenoma. The data were analyzed, and unweighted multi-rater kappa statistics were calculated.
Results: Four of the six participants' data were available at the time of this writing. The mean kappa value for all categories was 0.83 (almost perfect reproducibility). The kappa statistic for MVHP, GCHP, TSA, and adenoma was in the almost perfect category (0.81 - 1.00); whereas the SSA had a kappa value of 0.63 (substantial reproducibility). The kappa value when both types of hyperplastic polyps were measured against the remaining entities was 0.81 (almost perfect reproducibility). The results for all six participants will be presented.
Conclusions: Our results show that with well-defined diagnostic criteria and tutorial training, well-oriented examples of serrated colorectal polyps and adenomas can be diagnosed with substantial reproducibility by non-GI pathologists.

627 Prognostic Significance of Morphologic Features in Mucinous Colorectal Carcinomas

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Background: Colloid carcinomas, defined histologically as small numbers of epithelial clusters floating in abundant mucin, are distinct neoplasms with a relatively good prognosis in organs such as breast and pancreas, but have not been well characterized in the colorectum. Mucinous colorectal carcinomas (MCC), defined as tumors with >50% mucinous component, are histologically heterogeneous and have been reported to have a worse prognosis than non-mucinous adenocarcinomas in some but not all studies. We undertook this study to determine the clinical impact of various morphologic features of MCC.
Design: H&E stained sections from 142 cases of primary MCC (80M, 62F, mean age 66 yrs) were evaluated for the amount of mucinous component, cellularity and amount of signet ring cells (SRC) within the mucinous areas as well as growth pattern (clinging vs. floating epithelium, nodular vs. infiltrating mucin). Tumors with >50% SRC were classified as SRC-type. Non-SRC-type tumors showing a predominantly floating pattern with <50% cellularity were classified as colloid-type. Immunohistochemical staining for DNA mismatch repair (MMR) proteins hMLH1 and hMSH2 was performed in a subset of cases (n=91) and cases classified as MMR-normal and MMR-abnormal.
Results: The cases were classified as having a floating (26.8%), clinging (39.4%) or mixed (33.8%) pattern. Eleven cases were colloid-type (7.7% overall, 28.9% of cases with floating pattern). Twenty-two cases were SRC-type (15.5% of all cases, 50% of cases with floating pattern). No correlation was seen between cellularity or growth pattern and presence of distant metastases or survival, although floating pattern and higher cellularity were significantly associated with lymph node metastases ($p=0.001$ and 0.015, respectively). We were not able to document improved survival in MMR-abnormal cases. With a median follow-up time of 34 months, a worse disease-specific survival was seen in cases with an increased (>75%) mucinous component (log-rank test $p=0.04$) and in SRC-type MCC (log-rank test $p=0.002$). However, none of the patients with colloid-type MCC had lymph node metastases or died of their disease.
Conclusions: MCCs are histologically heterogeneous. SRC-type MCC and carcinomas with >75% mucinous component are associated with a worse prognosis compared to other types of MCC. Pure colloid-type carcinomas similar to those seen in other organs are rare in the colorectum. They appear to have a good prognosis, although this needs to be confirmed in larger studies.

628 Prognostic Relevance of Mucin Core Protein, beta-Catenin and p53 Expression in Mucinous Colorectal Carcinomas

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Background: Expression of mucin core proteins MUC1 and MUC2 (seen in 30-40% of cases) and aberrations of p53 and beta-catenin (seen in 55-80% of cases) have been shown to impact the prognosis of colorectal carcinoma (CRC). Only a limited number of studies have evaluated the significance of MUC1, MUC2 and p53 expression in mucinous colorectal carcinomas (mCRC), often with conflicting results, and beta-catenin expression has not been characterized in mCRC. We thus examined the expression of these proteins in mCRC, and assessed their prognostic implications as well as their relationship to defects in the expression of DNA mismatch repair (MMR) proteins hMLH1 and hMSH2.
Design: Immunohistochemical (IHC) staining for MUC1, MUC2, p53, beta-catenin, hMLH1 and hMSH2 was performed on 91 cases of primary mCRC (52M, 39F, mean age 67 yrs). Cases were considered positive if >10% cells expressed MUC1, MUC2, and nuclear beta-catenin or > 20% cells expressed nuclear p53. Cases lacking nuclear staining for either hMLH1 or hMSH2 were considered MMR-abnormal. Clinicopathologic features were recorded and correlated with IHC expression patterns.
Results: All cases expressed MUC2, while 36 (39.5%) expressed MUC1. Lymph node metastases were more frequent in MUC1 positive cases (50% vs 19%, $p=0.003$); however, there was no association between MUC1 expression and T stage, lymphovascular invasion, distant metastases or disease-specific survival (median follow-up 32 months). Nuclear beta-catenin expression was seen in 31 (34%) cases and did not correlate with any clinicopathologic variable. p53 expression was seen in 26 (28.6%) cases, was more frequent in left-sided carcinomas (40.5% vs 18%, $p=0.035$), but showed no association with other clinicopathologic variables. There were 24 (26.4%) MMR-abnormal cases that were more frequent on the right side (44% vs 7%, $p<0.0001$). Although MMR-abnormal cases showed less frequent MUC1, p53 and nuclear beta-catenin expression compared to MMR-normal cases, the differences were not statistically significant.
Conclusions: In contrast to non-mucinous CRC, mCRCs show a high frequency of MUC2 expression, a low frequency of aberrations in beta-catenin and p53 and no association between MUC1, p53 and nuclear beta-catenin expression with worse prognosis. These findings highlight important differences between mucinous and non-mucinous CRC in the molecular pathways that mediate tumor progression.

629 Lymphocytic Esophagitis in Pediatric Inflammatory Bowel Disease: A Manifestation of Upper Gastrointestinal Crohn Disease

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Background: Lymphocytic esophagitis (LE) is a recently described entity, which is histologically defined by marked esophageal lymphocytosis with no or only rare intraepithelial granulocytes. The original description of 20 cases included 8 cases (40%) of Crohn disease (CD), the majority of which were pediatric patients. This study was performed to evaluate histologic criteria for LE and to investigate the association between LE and pediatric CD.

Design: Blinded retrospective morphologic analysis was performed on upper and lower gastrointestinal biopsies from pediatric patients with well established clinical diagnoses of CD (N = 44) and ulcerative colitis (UC; N = 21), as well as non-inflammatory bowel disease (IBD) control patients with upper gastrointestinal (UGI) symptoms (N = 20). The upper endoscopies of IBD patients were performed regardless of UGI symptoms upon entrance into the University of Iowa Health Care system. The highest density of intraepithelial lymphocytes (IEL), neutrophils (IEN) and eosinophils (IEE) per high power field (hpf; 40X) were counted and 13 additional morphologic variables were analyzed. Independent blinded chart review was performed to confirm the clinical diagnosis and type of IBD, and to evaluate for symptoms referable to the UGI tract. LE was defined as >50 IEL/hpf and a ratio of >50:1 IEL to IEN and/or IEE.

Results: LE was identified in 13 of 44 (30%) patients with CD, and only 1 of 21 (5%) patients with UC and 1 of 20 (5%) non-IBD control patients; the difference was statistically significant when comparing CD verus UC (p-value = 0.0259), and CD versus non-CD (p-value = 0.0037). Five of the 44 (11%) patients with CD had esophagitis with granulomas, and two of these met the diagnostic criteria for LE. There was no difference in the incidence of UGI symptoms between the CD patients with LE (5 of 13; 38.5%) and the CD patients with esophagitis with granulomas (2 of 5; 40.0%: p-value = 1.00).

Conclusions: 1) The histologic diagnosis of LE is associated with pediatric CD and is a relatively common finding in this population (30%). 2) If LE is identified in patients with known CD, it is likely a manifestation of UGI-CD rather than esophagitis due to other etiologies or a variant of normal. 3) LE is not specific for CD, and this finding should not be considered diagnostic of IBD.

630 BD ProExC (ProExC) Immunostaining in Paget's Disease

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Background: BD ProExC is an immunocytochemical assay that targets the expression of topoisomerase II-alpha (TOP2A) and minichromosome maintenance protein-2 (MCM2), two genes shown to be overexpressed in HPV-induced high grade cervical and anal squamous intraepithelial lesions (SIL) and carcinomas. As a biomarker for high-risk HPV, ProExC immunostain is useful in the diagnosis of biopsies that are difficult to assess for high grade CIN or AIN with H&E stains alone. In selected cases, the differential diagnosis of SIL can include extramammary Paget's disease (EMPD), a type of intraepithelial carcinoma that is not known to be HPV-related. ProExC is a recently developed assay that has not yet been widely utilized. We recently noted immunoreactivity for ProExC in EMPD, a finding not previously reported.

Design: Twenty-two cases of Paget's disease (7 perianal, 7 vulvar, 8 mammary) were retrieved from our files. After slides were reviewed and diagnoses confirmed, immunostain for ProExc and appropriate controls were performed on serial sections of a representative formalin fixed block from each case. In-situ hybridization (ISH) for low-risk (LR) and high-risk (HR) HPV subtypes was performed on 12 of the EMPD including 4 cases in which PCR for HPV types 16 and 18 was also performed.

Results: Positive nuclear staining for ProExC was present in Paget cells in 100% of the cases irrespective of tissue site.Virtually all of the Paget cell nuclei stained positive in each case. ISH was negative for LR and HR HPV subtypes in all of the EMPD tested. PCR was positive for HPV 16 in one perianal case that was also p16+.

Conclusions: (A). Positive staining for ProExC is common in perianal, vulvar, and mammary Paget's disease.(B) Positive staining for ProExC in EMPD of the perineum constitutes a potential pitfall in the diagnosis of squamous intraepithelial lesions.

631 Morphologic Alterations of Esophageal Carcinomas Post Neoadjuvant Chemoradiation Therapy

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Background: This study aims to evaluate the morphologic changes in esophageal carcinomas induced by preoperative neoadjuvant chemoradiation therapy. We describe here previously unreported histopathologic alteration in these tumors.

Design: Thirty six consecutive cases of esophageal carcinoma with pre-operative neoadjuvant chemoradiation therapy from 2004 to 2007 were included. The biopsies were paired with their gastroesophagectomy specimens and the histomorphologic studies were performed.

Results: The patients were predominant male (30/36) with average age of 64.2 years (male) and 68 years (female). Residual carcinomas were present in 27/36 cases. Variable amounts of acellular mucin deposits, focal tumor necrosis, stromal fibrosis and chronic lymphohistiocytic infiltrates were noted in all cases. There were 25 cases sharing the similar histopathologic patterns present in their pre-treatment morphology. Five of thirty-six cases showed post-treatment morphologic alteration from conventional adenocarcinoma to squamous differentiation (1/36), clear cell pattern (1/36), adenoid cystic pattern (1/36), basaloid type (1/36), and small cell type (1/36).

Conclusions: Histopathologic changes have been occasionally observed in a variety of carcinomas after neoadjuvant chemoradiation therapies. The neoplastic cells can have different morphologic appearances, presumably due to metaplasia or dedifferentiation. The clinical and pathologic significance of these changes is largely unknown. It is imperative for practicing pathologists to recognize these unexpected morphologic changes to avoid a misdiagnosis.

632 t(11;18)(q21;q21) in Gastric Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma: A Molecular, Immunohistochemical and Histological Study of 65 Cases

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Background: Gastric MALT lymphoma is among the most common of extranodal marginal zone lymphomas and often responds to treatment by H. pylori eradication. However, a subset of gastric MALT lymphomas with t(11;18)(q21;q21) has been shown to be resistant to treatment by H. pylori eradication. With a newly developed real-time RT-PCR assay for t(11;18)(q21;q21) in formalin fixed, paraffin embedded tissue, we studied the molecular, immunohistochemical and histological aspects of 65 cases of gastric MALT lymphoma with the aim of further understanding the role of molecular testing in this disease.

Design: 65 cases diagnosed as gastric MALT lymphoma between 1997 and 2007 were selected. Real-time RT-PCR for t(11;18)(q21;q21) was performed on 65 cases. PCR for immunoglobulin heavy chain (IgH) gene rearrangement, immunostain (CD20, CD3, CD43, CD5, CD10, Bcl-2 and cytokeratin), and morphological evaluations were performed on the cases with material available.

Results: Of the 65 analyzed cases, 16 (25%) were positive for t(11;18) (q21;q21). Of these, 15 (94%) showed IgH gene rearrangement by PCR. Of the 49 t(11;18) (q21;q21) negative cases, 7 of 16 cases tested (44%) showed IgH gene rearrangement. Aberrant co-expression of CD43 was observed in 12 of 15 (80%) t(11;18) (q21;q21) positive cases and 16 of 30 (53%) t(11;18) (q21;q21) negative cases. t(11;18) (q21;q21) was predominantly found in cases with monocytoid morphology. 15 of 16 translocation positive cases (94%) were monocytoid, compared to 11 of 27 (41%) examined translocation negative cases. Cases with either plasmacytoid morphology or increased (>20%) large cells were usually t(11;18) (q21;q21) negative.

Conclusions: Our preliminary data show that t(11;18) (q21;q21) is most likely to be found in the subset of MALT lymphoma cases having monocytoid morphology and is infrequent in cases with plasmacytoid morphology or increased large cells. Most cases with t(11;18) (q21;q21) also have IgH gene rearrangement, and they are more likely to express CD43 than are translocation negative cases. More in-depth studies on the correlation between genotype and phenotype may further our understanding of this translocation and the clinical behavior of gastric MALT lymphomas.

633 Tumour Budding Is an Independent Predictor of Survival in Stage II, pT3N0 Colorectal Cancer

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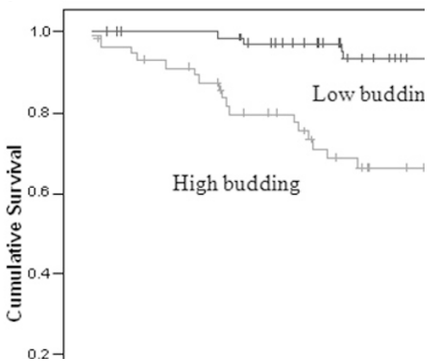
Background: Tumour budding, the detachment of tumour cells from adenocarcinomas at the invasive front, is thought to be an early step in the metastatic process. Few studies have examined the prognostic significance of tumour budding in Dukes B, pT3N0 colorectal cancer and a cut-off value for classifying into high and low risk groups based on H&E examination alone has not yet been determined.

Design: 128 patients with curative Dukes B, pT3N0 colorectal carcinomas between 1990-2004 were identified from our colorectal cancer database. Full clinical, pathological and follow-up data was available on each patient. Tumour budding was defined as isolated tumour cells or clusters of <5 at the invasive tumour front. All tumour slides were assessed for tumour budding (range 3-7 slides) and counts were generated from 5 areas at 20X HPF by 2 pathologists independently. Cases with disagreement were re-evaluated together using a double-headed microscope. A median bud score per patient was generated. Patients with median bud score of ≥1 were classified in the high budding group and those with median bud score=0 were in the low budding group. Data was statistically analysed and correlated with clinical and pathological features and patient outcome.

Results: There were 57 patients in the high budding group and 71 in the low budding group (mean age 71 vs 72). The median follow up was 5.7 and 3.5 years respectively. Using Wilcoxon analyses, high budding correlated with infiltrative tumour front ($p<0.01$), lymphovascular and neural invasion ($p<0.05$). By univariate analysis, patients with high budding had a worse prognosis ($p<0.001$). 36.8% cancer deaths occured in high budding group vs 11.3% in low budding group. Tumour budding was the only independent predictor of survival by multivariate analysis ($p\leq0.0001$). The hazard ratio was 4.76 (95%CI 2.09-10.85).

Conclusions: Tumour budding is easily assessed by conventional H&E analysis and can be used as a prognostic indicator in determining individualised treatment for pT3N0 colorectal carcinomas. A median bud score ≥1 is applicable in routine pathological assessment to identify the high budding group which is associated with a poorer outcome.



634 Diagnostic Yield for GVHD and Alternate Diagnoses in Biopsies from 1209 Endoscopies in Bone Marrow Transplant Patients

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Background: Bone marrow transplant (BMT) patients with gastrointestinal symptoms often have upper and lower endosocopies with biopsies to diagnose or exclude acute graft vs. host disease (aGVHD). We reviewed such biopsies to determine the diagnostic yield for aGVHD, and for other abnormalities that might explain symptoms.

Design: Histologic and endoscopic features of gastrointestinal tract biopsies from BMT patients (1995-2005) were compiled. Equivocal cases and alternate diagnoses were reviewed before classification.

Results: Biopsies from 617 upper endoscopies (UGI) and 592 lower endoscopies (LGI) were studied. The diagnostic yield for aGVHD was 41% for UGI biopsies, and 55.6% for LGI. In patients with both UGI and LGI biopsies, aGVHD was present in both in 40%, in only LGI in 13%, and only UGI in 9%. In UGI biopsies, duodenum yielded aGVHD in 40.1% of cases, stomach in 25.1%, and both in 22.4%. Duodenum was the only site involved in 17.8%; stomach was the only UGI site in 2.7%; esophagus was the only site involved in 2 cases. The yield for aGVHD in UGI was ~55% in the 6 weeks post-BMT, decreased to ~40% at 6 months, fell to ~28% thereafter. For LGI, the yield of aGVHD remained ~50% for a year or more post-BMT. Biopsies from endoscopically abnormal colonic mucosa were more likely to yield aGVHD (59%), but there was 38% yield from normal mucosa. Endoscopic findings most associated with colonic aGVHD were colitis or ulcers (68%). Other common pathologiic diagnoses in LGI: normal mucosa (26.1%), regenerative epithelial changes (3.9%), distortion/healing injury (3.4%); in the duodenum: normal mucosa (40%), regenerative epithelial changes (4.7%) distortion/healing injury (4.6%); in the stomach: normal mucosa (49.5%), chemical gastropathy (6.9%) gastritis (5.5%). Although a rare site for aGVHD, esophagus yielded 4 of the 8 cases of CMV, 4 candida, 3 HSV, 1 bacterial infection, and 36 ulcers. There were 3 cases of pseudomembranous colitis, 2 ischemia, 2 sprue, 1 cryptosporidiosis.

Conclusions: The diagnostic yield of aGVHD in UGI and LGI biopsies from BMT patients is excellent, 41% and 55.6%. This persists for LGI biopsies beyond a year post-BMT. Both endoscopically normal and abnormal mucosa should be biopsies, as both have significant yields of aGVHD. If aGVHD is not present, the likelihood of finding an alternate explanation for symptoms is low; most of the remaining biopsies are normal or nonspecific. Esophageal biopsies are unlikely to be diagnostic of aGVHD, but more likely to have CMV, HSV, or ulcers.

635 Evaluations of Diagnostic Markers of Dysplasia in Endoscopic Mucosal Resections of Barretts Mucosa

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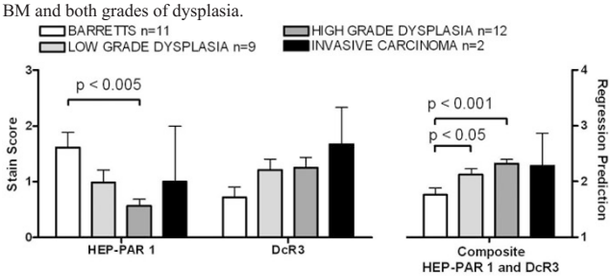
Background: There is considerable intra- and interobserver variability in the diagnosis of dysplasia in biopsies from Barretts mucosa (BM). Endoscopic mucosal resection (EMR) specimens from patients with BM provide an ideal resource to test candidate immunomarkers of dysplasia since they are large, well-oriented and optimally fixed. We tested several antibodies suggested by recent abstracts from other investigators as well as our own cDNA microarray study [Cancer Res 2005; 65: 3146].

Design: A tissue microarray was created from Hollandes fixed, paraffin embedded EMR specimens obtained from 20 patients with BM. A total of 147 tissue cores were included from areas graded as negative for dysplasia, LGD, HGD, and invasive carcinoma. Immunohistochemical stains for Hep-Par-1, amicar, S-100a2, and DcR3 were scored for staining intensity (0-3). Linear regressions were used to determine associations between histology and stain intensity. Students unpaired t test was used to test for differences between histologic groups.

Results: Statistical analysis revealed that intensity of S-100a2 and p504 reactivity were not associated with dysplasia grade. Hep-Par-1 staining, however, was inversely associated with histologic severity ($R^2 = 0.23$, $p < 0.01$). When grouped according to histology, there was a significant difference in stain scores for HEP-PAR-1 between BM and HGD. DcR3 staining was positively associated with histologic severity ($R^2 = 0.15$, $p < 0.05$). Despite a positive correlation, there was no significant difference in stain scores for DcR3 between individual histologic groups. A combined regression equation including Hep-Par-1 and DcR-3 stain scores explained more of the variance ($R^2 = 0.31$, $p < 0.01$). When grouped according to histology, the results of combined staining for HEP-PAR-1 and DcR3 allowed prediction of histologic differences between BM and both grades of dysplasia.



Conclusions: Intensity of Hep-Par-1 and DcR3 reactivity correlates with grade of dysplasia in Barretts mucosa. When used in concert these markers may improve the ability to grade Barretts dysplasia.

636 Utility of Alpha-Methylacyl-CoA-Racemase (AMACR) Immunohistochemical Expression in Distinguishing Dysplasia from Adenoma in Inflammatory Bowel Disease

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Background: Distinguishing polypoid dysplasia from sporadic adenoma in patients with inflammatory bowel disease (IBD) patients is often difficult, and there are significant differences in treatment of these patients depending on the diagnosis. Recently, AMACR has been shown to be overexpressed in both dysplastic and malignant gastrointestinal epithelium. Variable staining has been reported in adjacent non-neoplastic epithelium. This study sought to evaluate the utility of AMACR expression in neoplastic and non-neoplastic colonic epithelium in the distinction of polypoid dysplasia and sporadic adenoma in IBD patients.

Design: 41 colonic biopsies or resection specimens of IBD patients and 13 sporadic tubular adenomas of non-IBD patients were stained immunohistochemically to detect AMACR expression. The IBD specimens included 14 low grade dysplasias, 11 high grade dysplasias, 9 lesions endoscopically identified as polyps, and 7 colonic adenocarcinomas. Among the IBD-associated polypoid lesions, 4 were considered sporadic adenomas and 5 were considered polypoid dysplasia on the basis of whether or not the adjacent colonic mucosa showed evidence of involvement by IBD. Sections of non-neoplastic adjacent colonic mucosa were included in the analysis for all cases. The intensity of AMACR expression was scored as negative, weak or strong, and the distribution of staining was recorded as absent, focal or diffuse.

Results: Weak positive AMACR staining was seen in 4 of 14 low grade dysplasias (29%), 5 of 11 high grade dysplasias (45%), 8 of 9 polypoid lesion (89%), and 4 of 7 adenocarcinomas (57%). All of the IBD-associated sporadic adenomas and 3 of 5 polypoid dysplasias were AMACR positive. AMACR expression was identified in 9 of 13 sporadic adenomas (69%). The adjacent normal mucosa was positive and focal in 3 of the 9 polypoid adenomas, 3 of 13 tubular adenomas, and 2 of 7 adenocarcinomas and coincided with positivity in the adjacent neoplastic areas.

Conclusions: AMACR is expressed in IBD-associated dysplasia and sporadic adenomas. The intensity and pattern of expression are not useful for distinguishing dysplasia from adenoma in IBD patients. In addition, the relatively low frequency of AMACR expression in IBD-associated dysplasia suggests that AMACR staining may not useful in distinguishing dysplasia from non-neoplastic mucosa as suggested in the literature. The focality of positive staining suggests that AMACR immunohistochemistry may not be useful in the biopsy setting.

637 Colon Cancer Secreting Protein-2 Is Expressed in a Spectrum of Common Adenocarcinomas

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Background: We previously described from gene expression microarray experiments that Colon Cancer Secreted Protein-2 (CCSP-2) expression is markedly increased in colon cancer. We also identified this protein in blood of mice bearing human colon cancer xenografts. This secreted protein hence has potential utility as a biomarker for early detection of colon cancer and for monitoring of therapeutic responses. This study describes expression of CCSP-2 in colon cancer specimens and in an expanded list of common human cancers.

Design: Initial analysis of CCSP-2 expression across a spectrum of human cancers was done by hybridization of a CCSP-2 cDNA probe to a dot blot membrane containing RNA from cancer tissue and paired normal tissue across a variety of human cancers. To provide better sensitivity for analysis of CCSP-2 expression, we developed an immunohistochemical assay for CCSP-2 protein expression. FFPE sections from 9 types of common adenocarcinomas were stained with an antibody shown by Western blot analysis to be specific for CCSP-2.

Results: Dot Blot analysis detected increased expression in 70% of colon cancers (n=35) and 50% of rectal cancers (n=18).It also detected evidence of CCSP-2 induction in 30% of lung cancers (n=21); 20% of breast cancers (n= 51); 20% of endometrial cancers (n= 45); and 13% of ovarian cancers (n= 15). Immunohistochemistry results are presented in Table 1. All tumors in each subset classified as poorly differentiated did not stain for CCSP-2.

CCSP-2 Immunohistochemistry

Cancer Type	Number of Samples	% Positive
Colon	28	75
Endometrium	12	100
Lung	20	55
Stomach	12	42
Pancreas	28	39
Ovary	11	18
Breast	22	32
Esophagus	12	0
Prostate	9	22
[Pancreas Neuroendocrine]	11	0

Conclusions: These results demonstrate the expression of CCSP-2 in a variety of common human malignancies even though the function of CCSP-2 is completely unknown. The detection of CCSP-2 in blood of mice bearing human colon cancer xenografts known to be excretors of CCSP-2 holds out the promise of using this protein as a biomarker of colon cancer diagnosis and progression. The demonstration of CCSP-2 expression in other common tumors implies a basic function of the protein in other adenocarcinomas and its potential use as a biomarker in patients with these tumors.

638 Differentiating the Undifferentiated: Immunohistochemical Profile of Medullary Carcinoma of the Colon with an Emphasis on Intestinal Differentiation

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Background: Undifferentiated or medullary carcinoma (MC) is characterized by its distinct histologic appearance and relatively better prognosis compared to poorly differentiated colonic carcinoma (PDC). These two entities may be difficult to differentiate by light microscopy alone. Only limited immunohistochemical studies investigating MC have been reported. These studies suggest a loss of intestinal differentiation, exemplified by a high percentage of CDX2 negativity. Our aim was to further characterize the immunohistochemical profile of MC, with particular emphasis on intestinal markers.

Design: Paraffin blocks from 16 cases of MC and 40 cases of PDC were retrieved from the pathology archives at Rhode Island Hospital. Tissue microarrays were constructed and stained with an immunohistochemical panel including CK7, CK20, p53, intestinal trefoil factor 3 (TFF-3), chromogranin, synaptophysin, MSH-2, MSH-6, MLH-1, MUC-1, MUC-2 and calretinin.

Results: A significantly higher proportion of MC, as opposed to PDC, showed loss of staining for MLH-1 and for the intestinal transcription factor CDX2, in accordance with previous studies. MLH-1 staining was present in only 20% of MC cases compared with 58% of the PDC cases (p=0.016), whereas CDX2 was positive in 13% of MCs and 63% of PDCs (p=0.001). Interestingly, calretinin staining was strongly positive in 73% of MCs compared to only 10% of PDCs (p<0.0001). Evidence of intestinal differentiation by MUC-1, MUC-2 and TFF-3 staining was seen in 100, 73 and 47% of the MCs respectively. These three markers were frequently positive in many of the CDX2 negative MC cases.

Conclusions: Medullary carcinoma of the colon retains a significant degree of intestinal differentiation as evidenced by its high percentage of staining for MUC-1, MUC-2, and TFF-3. Calretinin, MLH-1 and CDX2 may help to differentiate MC from PDC of the colon.

639 Human Papillomavirus (HPV) 16 and 18 in Anal Intraepithelial Neoplasia (AIN) and Anal Squamous Cell Carcinoma (SCC) Detected in Tissue Biopsies

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Background: Human papillomavirus (HPV) infection is a necessary prerequisite for the development of AIN and carcinoma. Few studies have characterized the specific oncogenic HPV types involved. We have recently shown that most anal carcinomas are associated with HPV 16. This report characterizes the distribution of HPV 16 and 18 in surgical specimens with AIN (I-III) and in anal SCC.

Design: Eighty-five anal biopsy and resection specimens were retrieved from our files: negative for dysplasia (10), AIN I (18), AIN II (21), AIN III (14), and previously HPV typed anal SCC (20). DNA was extracted from paraffin sections per standard protocol. HPV 16 and 18 were detected by Invader 2 (Third Wave Technologies, Inc) type specific probes for HPV 16 and 18.

Results: All specimens negative for dysplasia were negative for HPV 16 and 18. HPV 16 was present in AIN I, AIN II, AIN III, and SCC in 22%, 29%, 79%, and 94% of specimens respectively (Table 1). HPV 18 was detected in a small minority of cases. Dual infection with both HPV 16 and 18 was seen in 6% of AIN I and 14% of AIN III.

Table 1: HPV 16 and 18 in Anal Intraepithelial Neoplasia (AIN) and Anal Squamoud Cell Carcinoma (SCC)

Histology (No.)	Negative for HPV 16 and 18	HPV 16 +	HPV 18 +	Both HPV 16 and 18 +
Negative for Dysplasia (10)	10 (100%)	0 (0%)	0 (0%)	1 (6%)
AIN I (18)	13 (72%)	4 (22%)	0 (0%)	1 (6%)
AIN II (21)	13 (62%)	6 (29%)	2 (9.5%)	0 (0%)
AIN III (14)	1 (7%)	11 (79%)	0 (0%)	2 (14%)
SCC (18)	1 (6%)	17 (94%)	0 (0%)	0 (0%)

Conclusions: HPV 16 is prevalent in AIN III and anal SCC. HPV 18 is rarely associated with AIN. The overwhelming presence of HPV 16 in AIN III and anal SCC strongly suggests that HPV 16 infections correlate with anal carcinogenesis. Therefore, patients may benefit from type specific screening for HPV 16 in low grade AIN.

640 Nuclear Beta-Catenin Labeling Implicates Perturbations of Wnt Signaling in the Pathogenesis of Some Sessile Serrated Adenomas

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Background: Sessile serrated adenomas (SSAs) have recently been recognized as an alternative pathway to colorectal neoplasia distinct from the traditional APC/Beta-catenin pathway. However, we have recently shown that a subset of SSAs has nuclear labeling for beta-catenin (Wu et al. AJCP 2007 *in press*). Our goal was to validate this finding and to further elucidate the role of beta-catenin in this type of colorectal polyp.

Design: We obtained paraffin-embedded samples of colorectal polyps, comprised of tubular adenomas (TAs), SSAs, and traditional serrated adenomas (TSAs) from the surgical pathology archives. All samples were reviewed and classified by the criteria of Torlakovic et al (AJSP 2003) and correlated with clinicopathologic features. Immunohistochemical labeling for beta-catenin was performed using standard methods, and scored for the presence and absence of nuclear labeling as well as the distribution of positive labeling in each polyp.

Results: A total of 51 polyps were collected from 51 patients with 41 right-sided polyps, 9 left-sided polyps, and 1 colonic polyp site unknown. The polyps included 15 TAs, 34 SSAs, and 2 TSAs. There was no significant difference in patient age or gender among each group. In normal mucosa, beta-catenin labeling was confined at the crypt bases (bottom 10%) and was seen as scattered positive nuclei admixed with membranous labeling. By contrast, abnormal labeling for beta-catenin was seen in 14/15 (93%) of TAs, in 9/34 (26%) sessile SSAs, and in 1/2 (50%) of TSAs. In TAs, nuclear labeling was present in >90% of cells of the polyp and the labeling was intensely positive. By contrast, nuclear labeling in SSAs involved ~50% of the cells, was of moderate intensity, and included patchy membranous labeling. Overall, this pattern was reminiscent of beta-catenin labeling of progenitor cells at the crypt bases in normal colonic epithelium. Sequencing for beta-catenin exon 3 in SSAs with nuclear beta-catenin labeling is ongoing, but the data was unavailable at the time of abstract submission.

Conclusions: These findings validate our previous report of nuclear beta-catenin labeling in a subset of colonic polyps morphologically classified as SSAs. Nuclear labeling in SSAs is unlike that seen in tubular adenomas in which nuclear beta-catenin occurs via mutations in the APC/beta-catenin. We propose that nuclear beta-catenin labeling in SSAs is a marker of an expanded progenitor cell population.

641 APC Mutation Frequency and Nuclear ß-Catenin Expression in KRAS Mutated Conventional and Serrated Colorectal Adenomas

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Background: APC mutations with disruption of the *Wnt* signaling pathway are associated with nuclear translocation of ß-catenin in conventional (APC) pathway colorectal carcinomas and their precursor lesions but are not predicted to occur within the serrated pathway. While the $BRAF^{V600E}$ mutation is a specific marker of the serrated pathway, precursor adenomas of both pathways may show KRAS mutations and exhibit overlapping histological features. This study tests the *hypothesis* that increased nuclear expression of ß-catenin distinguishes adenomas of non-serrated (conventional) and serrated histogenesis.

Design: KRAS codon 12, 13 mutations were assayed in a series of 55 serrated adenomas (SA) and 87 conventional adenomas (CoA). Data yielded a total of 13 KRAS mutated SA and 18 CoA. APC sequencing was performed on amplified DNA using 4 overlapping primer sets within exon 15 which encompasses the mutation cluster region (*mcr*). Immunohistochemistry (IHC) for ß-catenin was performed on formalin-fixed sections with mouse anti-ß-catenin monoclonal antibody from ZYMED-Invitrogen (San Francisco, CA) on all cases and 15 additional SAs with a $BRAF^{V600E}$ mutation. AutoCyte QUIC Immuno 1.2 software was used to quantify nuclear staining in 5 digitized fields with highest nuclear staining frequency per section and expressed as % of total epithelial nuclei positive.

Results: APC mutations were identified in 6 of 18 (33%) of KRAS-mutated CoA compared to 0 of 13 KRAS mutated SA (p <.05; Fisher Exact). Mean % nuclear ß-catenin positivity on IHC image analysis by adenoma category is listed below:

	SA (Total)	KRAS mut SA	KRAS wt SA	KRAS mut CoA
No. of Cases	28	13	15	16
ß-cat. mean±SE	10.0±3.3	17.9±6.5	4.9±2.0	22.3±5.3

KRAS mut CoA vs. KRAS mut SA, p=ns; KRAS mut CoA vs. KRAS wild type (wt) SA, p=.005; KRAS mut SA vs. KRAS wt SA, p=0.05.

Conclusions: 1) APC mutations were not found in serrated adenomas. 2) Nuclear expression of ß-catenin did not distinguish KRAS mutated adenomas of serrated vs. conventional (APC) pathway histogenesis. 3) Serrated adenomas with KRAS mutations showed increased nuclear ß-catenin relative to KRAS wild-type serrated adenomas.

642 Expression of Mineralocorticoid Receptor and 11β-Hydroxysteroid Dehydrogenase Type II in Stage II Colonic Cancer

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Background: One of the major physiological functions of the colon is water and electrolyte metabolism, a process regulated by mineralocorticoids. Aldosterone is the major endogenously secreted mineralocorticoid, which acts by binding to the mineralocorticoid receptor (MR) in colonic epithelium. The ability of non-selective MR to bind mineralocorticoids is mediated by the enzyme 11β-Hydroxysteroid Dehydrogenase Type II (11β-HSD2), which inactivates cortisol to cortisone, preventing binding of glucocorticoids by MR. Our goal was to determine the pattern of expression and prognostic value of MR and 11β-HSD2 in a cohort of TNM stage II colon cancer cases using tissue microarray technology.

Design: In this study, we retrospectively analyzed paraffin embedded microarray specimens from 133 consecutive patients with TNM stage II colonic carcinomas for MR and 11β-HSD2 expression by IHC. A recently described mouse MAb MR1-18 was used for MR; a rabbit polyclonal Ab H-145 was used for 11β-HSD2. The level of protein expression was scored semiquantitatively on a scale of 0-3+. MR and 11β-HSD2 expression were also quantitated by real-time RT-PCR.

Results: Expression of both MR and 11-βHSD2 was detected in normal colonic epithelium and colonic adenomas with a predominant nuclear localization for MR and cytoplasmic staining for 11β-HSD2. In colon cancer MR and 11β-HSD2 expression was observed in 71 (53.3%) and 80 (60%) of 133 cases. The staining patterns for MR and 11β-HSD2 were similar to those seen in normal colon. There was strong correlation between MR and 11β-HSD2 staining in individual cases (R=0.57, P<0.0001). Expression of MR and 11β-HSD2 was directly associated with lower tumor grade (P=0.02 and P=0.0001, respectively) and both of these markers were lost in higher grade tumors. Univariate analysis of survival revealed a significant direct correlation between MR expression and overall and disease free survival (P=0.02). Multivariate analysis indicated that loss of MR expression was an independent predictor of disease recurrence (P=0.03) and poor overall survival (P=0.04).

Conclusions: This study is the first to demonstrate expression of MR and 11β-HSD2 in colonic adenocarcinomas. MR and 11β-HSD2 expression correlates inversely with

degree of differentiation and may be involved in cancer progression. Determining MR expression in these tumors may provide important prognostic information and may represent a novel therapeutic target in colonic neoplasms.

643 Presence of Adenomatous Change in Invasive Colorectal Carcinoma Is a Favorable Prognostic Factor

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Background: Transition from normal epithelium to adenoma to invasive colorectal carcinoma is due to acquired accumulation of molecular defects, and is accompanied by recognizable histomorphologic features. Recent molecular studies have shown that at least two major pathways (chromosomal or microsatellite instability) are responsible for colorectal cancer. We hypothesize that the presence of low grade adenomatous change in invasive cancer may serve as a surrogate marker for the progression and potential prognostic factor for colorectal cancer.

Design: 259 consecutive surgical resections of primary invasive colorectal carcinoma were retrospectively reviewed. Based on the presence (group A, n=134) or absence (group B, n=125) of low grade adenomatous changes, the tumors were divided into two groups. Clinicopathologic parameters obtained for comparison analysis included: age and gender, tumor location and size, histologic type, tumor grade (low and high) and tumor TNM stage (according to AJCC 2002).

Results: There was no significant difference of age and gender between the two groups, although group A patients were slightly older (69.9 vs. 66.8, p=0.059). The tumors in group A occurred more frequently in the right colon (56.7%) than the rectosigmoid colon (32%). In contrast the tumors in group B occurred much more common in the rectosigmoid colon (63.2%) than the right colon (26.4%). For the depth of invasion, there were significantly more (49.3% vs. 28%) ≤pT2 stage tumors in group A than in group B ($p<0.001$). Overall the incidence of nodal metastasis in group A was significant less in group A (32.8%) than in group B (51.2%). The increased incidences of nodal metastasis were seen in all tumor stages. There were no difference in mean tumor sizes (4.1±2.7 vs. 3.9±2.4) and mean number of harvested lymph nodes (15.9 vs. 17.6).

Conclusions: Presence of low grade adenomatous change in invasive colorectal carcinoma is associated with low histologic grade of the cancer and lower stage at presentation. These tumors appear to occur in older age group and are associated more often with mucinous differentiation. Presence of adenomatous change is also associated with lower incidence of nodal metastasis. These results suggest the presence of low grade adenomatous change may indicate a less aggressive tumor and serve as a favorable pathologic prognostic factor.

644 Ruptured Appendiceal Mucinous Neoplasms: Prognostic Significance of Localized Extra-Appendiceal Mucin Deposition

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Background: Non-invasive appendiceal mucinous neoplasms (AMN) confined to the appendix are benign, whereas those with multifocal peritoneal mucinous deposits may progress to diffuse pseudomyxoma peritonei (PP) and follow an indolent but malignant course. AMNs may be associated with either cellular or acellular extra-appendiceal mucin limited to the right lower quadrant (RLQ). Although some investigators consider both of these situations to pose no, or very low, risk for recurrent or metastatic disease, the biologic importance of localized extra-appendiceal mucin has never been evaluated.

Design: We retrospectively identified 50 patients with AMNs and localized extra-appendiceal mucin confined to the RLQ, who also had >6 months of clinical follow up (mean: 49 months, range: 6-180 months). Representative tissue sections from the appendix were submitted in 19 (38%) cases and 31 (62%) appendices were completely submitted for histologic evaluation. Each case was evaluated for the amount of extra-appendiceal mucin (minimal or copious), degree of mucin cellularity, and clinical outcome.

Results: Extra-appendiceal mucin was acellular in 41 (82%) AMNs and contained tumor cell clusters in 9 (18%) cases (range: 1-12 cell clusters). Two of 41 (5%) patients with copious acellular mucin surrounding the appendix developed diffuse PP, but neither had their appendix entirely submitted for histologic evaluation. In contrast, 3/9 (33%) patients with tumor cells outside the appendix developed diffuse PP, one of which died of disease (p=0.04). None of the AMNs with acellular extra-appendiceal mucin that were submitted completely for histologic assessment progressed to diffuse PP, recurrent disease, or caused the death of the patient upon follow-up.

Conclusions: Patients with AMNs and extra-appendiceal acellular mucin localized to the RLQ are very unlikely to develop diffuse PP and do not die of their disease. However, complete microscopic examination of the entire appendix is necessary to exclude the presence of tumor cells outside of the appendix and is important for accurate prognostication. AMNs with cellular extra-appendiceal mucin are more likely to recur as diffuse PP and result in death of the patient, even if the mucin is paucicellular and confined to the RLQ.

645 Correlation of H&E Features, Immunohistochemical Expression of Neuroendocrine Markers, and Microsatellite Status in 149 Colorectal Carcinomas

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Background: The identification of neuroendocrine features (NEF) in colorectal carcinomas (CRC) on H&E stains often triggers additional immunohistochemical work-up for neuroendocrine markers (NEM). Given the different therapeutic approach and prognosis, it is necessary to classify a tumor as a neuroendocrine carcinoma (NEC) or a carcinoma with neuroendocrine differentiation. We evaluated NEM expression in CRC to determine the frequency and pattern of staining in typical adenocarcinomas and in tumors with NEF, and correlated the findings with microsatellite instability (MSI) status.

Design: Previously constructed tissue microarrays (2 cores per case) containing 149 CRC were stained with H&E, and antibodies directed against Chromogranin, Synaptophysin, CD56 and monoclonal NSE. Tumors had been previously assessed for MSI status. H&E sections were evaluated to determine the presence of NEF. Pattern of expression (focal or diffuse) and intensity (weak or strong) of NEM were scored.

Results: The immunoreactivity for individual and combined NEM is shown in the table. NEF were seen in 19 of 149 (13%) CRC. Of these 19, 9 were negative for all NEM. One (a rectal tumor) showed diffuse staining with all NEM. Two were positive for chromogranin (one focal, one diffuse). One was diffusely positive for synaptophysin and one had focal NSE expression. Sixty of the 149 CRC (40%) had at least one positive NEM. Of the 50 CRC with a positive NEM, but no NEF, 20 were positive for chromogranin (19 focal, 1 diffuse), 27 were positive with NSE (25 focal, 2 diffuse), and 4 with CD56 (focal). MSI status was available in 59 of the 60 NEM positive cases. 35 were MSI stable and 24 were MSI unstable. NSE immunoreactivity was more often seen among the MSI unstable cases.

Conclusions: Expression of NEM in typical CRC is rare except for occasional scattered crypt cells that are chromogranin and CD56 positive. The combined and diffuse expression of NEM in carcinomas that exhibit NEF on H&E support a diagnosis of NEC. NEF on H&E sections are subjective and lack correlation with NEM expression. NSE immunoreactivity was most often seen in MSI unstable cases.

NEM in CRC and Association with NEF and MSI Status

NEM (+/total CRC)	NEF on H&E/+NEM	MSI unstable/+NEM
Synaptophysin (2/136)	2/2	0/2
Chromogranin (23/135)	3/23	7/22
CD56 (5/131)	1/5	4/5
NSE (30/120)	3/30	13/30
All 4 NEM (1/136)	1/1	0/1
Chromo+CD56 (1/136)	0/1	1/1
Chromo+NSE (4/136)	0/4	1/4
CD56 +NSE (1/136)	0/1	1/1

646 Gastric Carcinomas with Lymphoid Stroma Show Distinct Molecular Features That Reflect Their Epstein-Barr Virus (EBV) and Microsatellite Instability (MSI) Status

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Background: Gastric carcinoma (GC) with lymphoid stroma, variably termed "medullary" and "lymphoepithelial" carcinoma, is characterized by syncytia of tumor cells enmeshed within a mononuclear cell-rich stroma. Although these tumors have not been extensively studied, they often show evidence of EBV infection or MSI. The aim of this study was to better define the morphologic and molecular features of GCs with lymphoid stroma, and to determine whether they vary with respect to EBV and MSI status.

Design: Tissue sections from 20 consecutive GCs with lymphoid stroma were subjected to *in situ* hybridization for EBV-encoded RNAs (EBER) and assessed for MSI. Each case was evaluated for pathologic stage, extent of glandular differentiation, and vascular invasion. Immunostains were also performed to characterize the tumors (MLH1, MSH2, MGMT, bcl-2, β-catenin, p53, p27, p21, p16, E-cadherin) and the associated inflammatory infiltrates (CD20, CD3, CD4, CD8 and CD56).

Results: The 20 tumors fell into 3 mutually exclusive groups: EBV+/MSS (n=6), EBV-/MSI-H (n=7), and EBV-/MSS (n=7). One-third of EBV+ GCs showed glandular differentiation, versus 64% of EBV- tumors (4/7 MSI-H and 5/7 MSS, $p>0.05$ for both comparisons). Seven (100%) MSI-H GCs showed loss of either MLH1 or MSH2, and bcl-2 expression, compared to 0% of EBV+/MSS and 0% of EBV-/MSS GCs ($p<0.005$ for all comparisons). Most (67%) EBV+ GCs showed loss of MGMT and 50% showed nuclear β-catenin staining (p=0.07 compared to EBV- groups), whereas increased p53 staining was present in 57% of EBV-/MSS, 17% of EBV+, and 0% of MSI-H GCs. All of the cases contained a CD3+/CD8+ lymphocyte-rich infiltrate, but CD56+ cells were significantly more numerous in EBV+ (100%) than MSI-H GCs (14%, p=0.04).

Conclusions: One-third of GCs with lymphoid stroma are EBV-/MSS and the remainder show mutually exclusive EBV positivity and MSI with near-equal frequency. These tumors are histologically indistinguishable, regardless of their pathogenesis. However, EBV+ tumors show loss of MGMT and nuclear accumulation of β-catenin, which may reflect DNA hypermethylation; and they contain numerous CD56+ inflammatory cells, presumably reflecting NK cells associated with virally infected tumor cells. In contrast, MSI-H GCs show loss of MLH1 and uniformly express bcl-2, possibly reflecting altered cell cycle regulation in these tumors.

647 Primary Leiomyosarcoma of the Pancreas: 9 Cases

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Background: A variety of mesenchymal tumors, including primary sarcomas of the pancreas, have been described, mostly as single case reports.

Design: A SNOMED search of the Mayo Clinic surgical pathology files from 1994 to 2006 identified 69 primary mesenchymal tumors of the pancreas; 54 of them were sarcomas. While this accounts for approximately 1.8% of total pancreatic tumors diagnosed during the study period, the majority of our cases were seen as extramural consultation material, so the true prevalence of primary sarcomas is probably much lower. Leiomyosarcoma is the most common primary pancreatic spindle cell tumor in our files (22/54, 40.7%, see Table 1) followed by gastrointestinal stromal tumor (14/54, 25.9%). Nine patients with pancreatic leiomyosarcoma diagnosed and treated at our institution are described.

Results: There were five males and four females; mean age = 63 years, range 39-87 years. The patients presented with abdominal pain, weight loss, and jaundice for an average of 4.3 months. Seven tumors were located in the head and two in the tail; the average size of tumor was 4.7cm (1.0–12.5 cm). The histology of the nine primary pancreatic leiomyosarcomas (7 spindle and 2 epithelioid) was identical to leiomyosarcomas of other sites. An immunohistochemical battery including smooth muscle actin, desmin, CD117 and CD34 confirmed the diagnosis. Pancreatico-duodenectomy was performed in four cases; three patients had palliative procedures and two had biopsies only. No lymph node metastasis was identified in the four resected tumors, but liver metastases were present in 4/9 patients. All nine patients died and five deaths were known to be disease-related. The overall mean survival was 31 months (ranging from 5-98 months).

Conclusions: Primary pancreatic leiomyosarcoma is the most common primary pancreatic sarcoma, though primary pancreatic sarcomas were exceedingly rare overall. Pancreatic leiomyosarcoma occurs in older adults with no gender preference. It is often located in pancreatic head and more likely to metastasize to liver but not regional lymph nodes. The prognosis is poor with a mean survival of 31 months.

Table1. 54 primary pancreatic sarcomas at Mayo Clinic (1994-2006)

Diagnosis	Number of cases
Leiomyosarcoma	22 (40.7%)
Gastrointestinal stromal tumor	14 (25.9%)
Undifferentiated high grade pleomorphic sarcoma	7 (12.5%)
Spindle cell sarcoma, NOS	7 (12.5%)
Liposarcoma	2 (3.7%)
Malignant peripheral nerve sheath tumor	1 (1.9%)
Ewing sarcoma	1 (1.9%)
Total	54 (100%)

648 Calretinin Is a Valid Marker To Assist Diagnosis of Hirschsprung Disease (HD) in Rectal Suction Biopsies without Use of Acetylcholinesterase (Ach) Staining

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Background: Varying success has been reported for the identification of HD using Ach enzymatic stains. Also frozen tissue for such stains causes artifact on permanent sections and sacrifices limited biopsy tissue. A recent study, using 10 full thickness of colon with HD, reported positive staining of calretinin in ganglion cells and their nerve trunks in normally innervated colon, but negative staining in hypertrophic nerve trunks (HNT) in HD segmments (J Clin Pathol 2004;57:712-716). The current study was to test validity of calretinin in assisting diagnosis of rectal suction biopsies and to compare it with neural specifc enolase (NSE) and vasoactive intestinal peptide (VIP).

Design: Over past 9 months, 22 pediatric patients (2 weeks old to 5 years old) underwent rectal suction biopsies. In each patient, there were one to three biopsies labeled "3 to 5 cm". Each biopsy was serially sectioned into 6 levels with H&E staining for light microscopy. The levels with either ganglion cells (16 controls) or HNT (6 HD cases) were destained and immunohistochemically re-stained for calretinin, NSE and VIP. Full thickness sections of pull-through colonic specimens of 6 patients, with biopsy diagnoses of HD, were also stained using the 3 markers.

Results: All 16 control cases showed adequate biopsies with ganglion cells (including immature ganglion cells in neural units) in at least one biopsy; two out of 16 cases (2/16) had one biopsy at 3 cm lacking both ganglion cells and hypertrophic nerve trunks (possibly from distally physiological hypo-ganglion zone), but ganglion cells were found at 5 cm in the two cases. Cytoplasmic and nuclear stains of calretinin were positive in all ganglion cells (16/16 controls) but negative in HNT (6/6 HD). This staining pattern was confirmed in all 6 pull-through colonic specimens (6/6). In addition, there was positive calretinin staining in mucosal nerve branches of 15/16 controls but in none of HD cases (biopsies or surgical specimens). NSE and VIP stains were positive in both ganglion cells and HNT.

Conclusions: We confirmed the role of calretinin in HD (as cited) and found that the mucosal calretinin expression was correlated with the presence of ganglion cells. Using this method, we propose to have 3 categories of adequate rectal suction biopsies: No HD (ganglion cells present), HD (no ganglion cells but HNT negative for calretinin) and non-diagnostic (no ganglion cells or HNT).

649 Expression of Cell Cycle Proteins p53, p21, p27, p14 and p16 and Proliferation Index Ki67 in Gastric Carcinomas

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Background: Gastric carcinomas (GC) are devastating and aggressive human tumors and molecular pathogenesis has been under intense investigation as a part of the effort to develop more effective therapeutic strategies for these tumors. This study investigates the role of the expression of 5 essential cell cycle molecules (p53, p21, p27, p14 and p16) and proliferating index (Ki67) as prognostic indicators in GC.

Design: The study included 116 gastrectomy specimens obtained from 116 patients with gastric cancer. Seven tumors were TNM-stage I, 35 II, 56 III, and 18 IV, whereas 92 tumors were low-grade (grade I and II intestinal type adenocarcinomas) and 18 high-grade (grade III intestinal type, and diffuse type adenocarcinomas). Formalin-fixed, paraffin-embedded 4µm sections were subjected to immunohistochemistry (streptavidin-biotin peroxidase) using monoclonal and polyclonal antibodies for p53, p21, p27, p14, p16, and Ki67. Results were expressed as % of positive cells. Mean follow-up time was 45.3 months (range 3.5-140 months).

Results: P53, p14, p16, p21 and p27 were detected in: 56%(65/116), 70%(81/116), 65%(75/116), 48%(56/116), and 82%(72/116) of the cases, respectively. Mean index for Ki67 was 25.34±7.3. High grade tumors exhibited higher indices for Ki67 (p=0.004), p53 (p=0.017) and p21 (p=0.03) compared to low grade tumors, whereas p14 and p16 were more frequently present in low grade tumors (p=0.001 and p=0.025 respectively). Ki67, p53 and p21 were more frequently expressed in advanced stage tumors (p=0.002, p=0.014 and 0.0012 respectively). Lower disease-free survival was correlated with **a)** high grade (p=0.0007), **b)** advanced stage (p=0.0008), **c)** higher Ki67 index (p=0.0075). p53 expression was associated with lower disease-free survival only in high grade p21(+) tumors (p=0.021). Spearman rank analysis revealed direct correlation between p21 and p27 (r=0.31562, p=0.00927) and p53 and p21 (r=0.5516, p=1.3079E-6). Finally, Cox regression analysis revealed that tumor grade, stage, Ki67 and p53 index were independent prognostic factors (CI: 0.032-0.502, p=0.03, CI:1.167-5.408, p=0.019, CI: 1.006-1.057, p=0.016, CI:1.000-1.0044, p=0.028).

Conclusions: The study shows that in cases of gastric carcinomas, tumor grade and stage and Ki67 and p53 indices are independent predictors of the outcome of the patients. p53 was associated with poor prognosis only in tumors overexpressing p21; that means high levels of p21 in tumor cells associated with aberrant p53 protein expression, may result in tumor recurrence.

Genitourinary

650 Hypermethylation of Tumor Suppressor Gene CpG Islands in Small Cell Carcinoma of the Urinary Bladder

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Background: Small cell carcinoma of the urinary bladder (SCBC) is a rare tumor which shows a common clonal origin with urothelial carcinoma. It bears a high metastatic potential, even when discovered in a localized state. Identifying the molecular underpinnings of this disease may elucidate useful clinical information regarding prevention, diagnosis, prognosis, treatment, and surveillance. DNA methylation is widely recognized as having a pivotal role in the process of carcinogenesis, but has not been explored in this pathology.

Design: We used quantitative methylation-specific PCR (qMSP) to analyze the DNA methylation status of four frequently hypermethylated tumor suppressors in small cell and transitional cell carcinoma (TCC) arising concomitantly in thirteen patients.

Results: We identify frequent methylation of *RASSF1* and *MGMT* and infrequent methylation of *MLH1* and *DAPK1* in cases of concomitant TCC and SCBC. Similar rates of methylation were found in pure and concomitant histopathologies with the exception of *MGMT*, which was much less frequently methylated in pure TCC.

Conclusions: These findings suggest that small cell bladder carcinoma and transitional cell carcinoma have common origins, establish DNA methylation of some tumor suppressors as frequent occurrences in both histopathologies, and suggest that *MGMT* methylation may be a SCBC-specific epimutation.

651 Germ Cell Origin of Testicular Carcinoid Tumors

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Background: Carcinoids are neuroendocrine tumors and most frequently occur within tissues derived from the embryonic gut. These tumors can occur in any organ site, but are rare in the testis. The cell type giving rise to testicular carcinoid is unknown. We hypothesized that testicular carcinoid may have a germ cell origin.

Design: We analyzed protein and genetic markers in four testicular carcinoid tumors using immunohistochemistry and fluorescence *in situ* hybridization methods.

Results: All four cases of testicular carcinoid tumor arose in a background of mature teratoma. Isochromosome 12p was identified in carcinoid tumor cells in all four samples. 12p overrepresentation was also observed in 3 cases. Isochromosome 12p and 12p overrepresentation were present in cells of co-existing mature teratoma in 3 cases. Carcinoid tumors showed strong immunoreactivity for synaptophysin and chromogranin; but no immunoreactivity for OCT4, CD30, c-kit, TTF-1, and CDX2. Membranous and cytoplasmic staining for beta-catenin was detected in 3 cases.

Conclusions: We found that the classic genetic alterations that characterize germ cell tumors, 12p isochromosomy and overrepresentation, are also demonstrable in testicular carcinoid tumors. These tumors showed uniform immunohistochemical expression of neuroendocrine markers, but lacked expression of CD30, OCT4, CDX2, TTF-1, and c-kit. Our findings suggest that testicular carcinoid is a phenotypic expression of teratoma and is of the same germ cell origin, rather than being derived from Leydig cells, as suggested by others.

652 Immunohistochemical Profile of Primary Urethral Carcinomas: Analysis of 60 Cases of Different Tumor Types

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Background: Primary carcinomas of the urethra are distinctly rare tumors and account for <1% of urinary tract malignancies. Because of the rarity of these tumors, their immunohistochemical profile has not been fully described. Goal of our study is to define the immunohistochemical profile of 60 cases of primary urethral carcinoma.

Design: 60 cases of primary urethral carcinoma (24 squamous cell carcinomas [SCC], 6 urothelial carcinomas [UC], 8 adenocarcinomas, 10 mixed carcinomas [MCa], 11 carcinoma not otherwise specified [CaNOS] and 1 lymphoepithelioma-like carcinoma) were evaluated. Secondary involvement of the urethra was clinically excluded. Formalin-fixed, paraffin-embedded tissue sections were used for immunohistochemistry. All