Gastrointestinal

470 Clinical and Pathologic Features of Ulcerative Colitis in Primary Sclerosing Cholangitis

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Background: The histologic features of ulcerative colitis (UC) in patients with primary sclerosing cholangitis (PSC) are, essentially, unknown. One previous study suggested that UC in patients with PSC is associated with a high rate of rectal sparing and backwash ileitis; however, microscopic examination of tissue specimens was not performed. This is the first study to systematically evaluate the histopathologic characteristics and distribution of UC in PSC patients and to compare the results to a control group of UC patients without PSC.

Design: 42 PSC patients with UC and 43 matched non-PSC UC controls were identified from the files of three hospitals between 1989 and 2005. Clinical, endoscopic and follow-up data (including incidence of pouchitis and pouch failure) were evaluated, and a detailed pathologic evaluation of biopsies (30 and 31 for PSC and non-PSC, respectively) or resection specimens (12 total colectomies for each group) was performed on all patients.

Results: In PSC patients, the median age at IBD diagnosis was significantly lower (23.5 yrs vs. 28.0 yrs, $p = 0.03$) and the percentage of patients undergoing colectomy for dysplasia (58.3% vs. 0%, $p = 0.004$) was significantly higher compared to controls. UC preceded the diagnosis of PSC for the majority of patients (57.8%). Pathologically, a significantly higher proportion of PSC patients had pancolitis (92.8%) versus controls (65.1%, $p = 0.002$), but the prevalence of rectal sparing [based on evaluation of resection specimens and biopsies (12.5% vs. 2.6%, $p = 0.17$)], and the prevalence of "backwash" ileitis (35% vs. 12.5%, $p = 0.24$) did not differ significantly between PSC and non-PSC patients. None of the PSC or non-PSC colectomies evaluated showed rectal sparing. Severity of disease, patchiness, appendiceal involvement, and the incidence of pouchitis upon follow-up (50% vs. 25%, $p = 0.40$) were not different between PSC and non-PSC patients. None of the patients had pouch failure.

Conclusions: The pathologic features of UC in patients with PSC are similar to those without PSC. Contrary to previous reports, PSC patients with UC do not show an increased rate of rectal sparing, backwash ileitis or the development of pouchitis upon follow-up.

471 Clear Cell Gastric Adenocarcinoma: A Distinctive Tubulopapillary Variant

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Background: Gastric cancers containing a significant population of clear cells are rare. The purpose of this study was to explore the incidence and clinicopathologic characteristics of gastric cancers with a minimum of 10% of the total tumor cell population composed of clear cells.

Design: One hundred and sixty one gastrectomy specimens over an 8-year period were retrieved and cases containing a minimum of 10% clear cells were included in the study. All relevant demographic and pathologic data were accessed. In addition to PAS and PASD stains, immunohistochemistry for the following antibodies was also performed: CK7, CK20, CDX2, CEA, a-feto protein (AFP), cyclin D1 and E-cadherin.

Results: Twelve cases contained from 10% to 90% clear cells. There were 9 males and 3 females ranging in age from 62 to 82 years (mean: 71.4 years). Eight cases were located in the gastro-esophageal junction, 2 in the cardia and 2 in the pyloric/pre-pyloric region. The tumors ranged in size from 2 to 9 cm (mean: 4.5cm). Grossly, all tumors were polypoid, exophytic lesions. All 12 cases had a tubulo-papillary pattern with varying proportions of tubules and papillary structures. The clear cells were PAS positive and this was sensitive to PASD, indicating the presence of glycogen. The extent of clear cell change was as follows: 90% clear cells (2 cases), 80% (2), 60% (1), 50% (1), 30% (3), 10% (3). Four cases were T1 tumours, 2T2, 5T3, 1T4; 8/12 had lymph node involvement. Eight cases showed CK7 positivity, 5 CK20 immunoreactivity, while all cases were CEA, cyclin D1, E-cadherin and CDX2 positive. All cases were AFP negative. Five patients had died of disease with 2 years of diagnosis.

Conclusions: Gastric cancers containing clear cells are commoner in males, have a predilection for the gastro-esophageal junction, have a polypoid gross appearance, are characterized by a tubulo-papillary pattern and retain E-cadherin expression but over-express cyclin D1. Despite their well-differentiated histologic appearance, this subset of adenocarcinoma appears to behave aggressively.

472 Anal Carcinoma and HPV in Immunocompetent Individuals

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Background: The HIV pandemic has led to a significant increase in the incidence of anal squamous cell carcinoma (SCC). Homosexual males are at increased risk for HPV infections and subsequent anal intraepithelial neoplasia (AIN) and SCC. Immune suppression and persistence of HPV infection in HIV infected individuals is believed to promote rapid progression to SCC. However, little is known concerning HPV and anal carcinoma in immunocompetent individuals. This study was performed to examine the incidence of HPV infection in anal carcinoma of immunocompetent patients.

Design: 20 cases of anal carcinoma were retrieved from the files of the All India Institute of Medical Sciences: 19 of the carcinomas arose in males, 1 in a female. Patients' ages ranged from 38 to 78 years (mean 52; median 49). All patients were HIV uninfected. Histologic diagnoses were SCC in 18 cases and adeno- and cloacogenic carcinoma in 1 instance each. DNA was extracted from a representative block of each of the formalin-fixed paraffin embedded tumors. The presence of HPV DNA in extracts was determined by nested polymerase chain reaction (PCR) using the MY09/MY11 outer primer and GP5+/GP6+ inner primer sets. The presence of HPV types 16 and 18 was determined using type specific PCR (primer sets directed to the E6/E7 region and L1 genes). Appropriate positive and negative controls were run.

Results: The mean age of 52 years in this cohort is consistent with that described previously for immunocompetent individuals (mean age for HIV+ men with anal cancer is 36). 17 of the cases were positive for HPV by the nested PCR. 14 were positive for HPV 16. One case that was negative for HPV by nested PCR was positive for HPV16, therefore, in total HPV DNA was amplifiable in 18 cases. All 20 cases were negative for HPV 18. The adenocarcinoma was negative for HPV, while the cloacogenic carcinoma was positive for HPV 16.

Conclusions: Anal carcinomas in immunocompetent individuals: (a) are associated with HPV infection in 90% cases (b) HPV 16 is the most common subtype, being present in 70% cases (c) are not associated with HPV type 18 and (d) may be preventable with vaccination for HPV type 16.

473 Gastric Spindle Cell Sarcomas May Not Be Gastrointestinal Stromal Tumors: Report of Three Cases of Primary Gastric Synovial Sarcoma with Molecular Confirmation

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Background: Mesenchymal tumors account for approximately 2% of all gastric malignancies. Of these, gastrointestinal stromal tumors (GIST) are by far the most common, followed by tumors with smooth muscle or neural differentiation. Synovial sarcoma (SS) is exceedingly rare as a primary gastric neoplasm and only a single case, cytogenetically characterized, has been reported. Herein, we report three cases of primary gastric synovial sarcoma with cytogenetic and molecular characterization.

Design: Three cases of primary gastric SS were identified at the Mayo Clinic and Yale New Haven Hospital. Two were initially diagnosed as GIST. Immunohistochemical studies for KIT, smooth muscle actin, muscle specific actin, desmin, cytokeratins AE1/AE3 and CAM 5.2, and EMA were performed in each case. Two tumors were characterized at the molecular level using fluorescence in situ hybridization (FISH) for rearrangements of the *SYT* locus and reverse transcriptase polymerase chain reaction (RT-PCR) for the two most common synovial sarcoma fusion genes (*SYT-SSX1* and *SYT-SSX2*). The third case was studied by standard cytogenetic analysis.

Results: All tumors occurred in adults (43-67 years) and ranged in size from 0.8-7 cm. Histologically, each showed a monophasic growth pattern, expressed cytokeratins and EMA, and were negative for all other markers tested, including KIT. Two cases harbored the *SYT-SSX2* fusion transcript; the other contained the chromosomal translocation t(X;18)(p11;q11.2), but determination of the specific type of fusion gene could not be performed. All tumors were treated surgically and two recurred locally within 1 year.

Conclusions: Primary gastric synovial sarcoma is rare but given the morphologic variation that can be seen in GIST, it should be considered in the differential diagnosis when faced with a KIT-negative spindle cell neoplasm. Interestingly, the three tumors in this series and the previously reported case occurred in older patients. Whether these tumors are rare or merely under recognized remains to be determined. Similar to synovial sarcoma of the extremities, primary gastric synovial sarcoma exhibits an aggressive biologic behavior and is resistant to imatinib mesylate therapy. Therefore, awareness of synovial sarcoma as a potential diagnostic pitfall in gastric spindle cell neoplasms is important.

474 Increased Expression of Nuclear Factor-kappaB/p50 Correlates with Disease Progression and Prognosis in Colorectal Adenocarcinoma

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Background: The nuclear transcription factor nuclear factor-kappa B (NF-kB) has been found to play a role in cancer development and progression through its influence on apoptosis. The aim of this study was to determine whether NF-kB is constitutively activated in colorectal cancer tissues and to determine the role of NF-kB in colorectal cancer development and tumor progression.

Design: Immunohistochemical staining was performed using the p65 and p50 subunits of NF-kB in normal epithelial, adenomatous and adenocarcinoma tissues. To identify the correlations between clinicopathological parameters and the survival, expression of both the NF-kB/p65 and NF-kB/p50 was assessed in 210 consecutive patients with colorectal carcinomas who had undergone colectomy or polypectomy due to intramucosal carcinoma arising from pre-existing adenomas.

Results: The immunoreactivity of both the NF-kB/p65 and NF-kB/p50 was significantly increased in the transition from normal mucosa and adenoma with low dysplasia to adenocarcinoma ($p<0.05$). Among the 210 cases of adenocarcinoma, NF-kB/p50 immunoreactivity was significantly correlated with the angiolymphatic invasion ($p< 0.01$), lymph node metastasis ($p<0.05$), liver metastasis ($p<0.05$), distant metastasis ($p<0.05$), and increasing stages of the Dukes' classification ($p<0.05$). On the other hand, well-differentiated adenocarcinoma is showed significantly higher expression of NF-kB/p65 than moderate and poorly differentiated adenocarcinomas ($p<0.05$). Importantly, the patients with expression of NF-kB/p50 (but not of NF-kB/p65) had significantly worse prognosis (Log-rank test, $p=0.001$). On multivariate analysis, Dukes' stage ($p=0.001$) and NF-kB/p50 expression ($p=0.003$) were independent prognostic indicators.

Conclusions: These results suggest that, both the NF-kB/p50 and NF-kB/p65 play an important role in colorectal tumorigenesis. Overexpression of NF-kB/50 is showed aggressive clinical biology and poor outcome; thereby suppression of NF-kB may be a useful therapeutic strategy for a subset of patients with colorectal carcinoma.

475 Tissue Microarrays Provide a Cost-Effective Method for Screening Colorectal Carcinomas for Lynch Syndrome

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Background: Immunohistochemical (IHC) stains for the mismatch repair (MMR) proteins help screen for Lynch Syndrome and identify microsatellite unstable colorectal carcinomas, providing prognostic information. It has been suggested that colorectal carcinomas be screened routinely for a MMR defect. Tissue microarrays (TMA) are a high-throughput method of analyzing multiple tumors and may make screening economically feasible. We hypothesized that TMAs, when used with IHC, are cost-effective and reliably representative of MMR protein expression in colorectal carcinomas.

Design: One hundred and sixty-eight colorectal carcinomas from the Columbus area Lynch Syndrome study were retrieved from the archival files, on which IHC for the MMR proteins had been previously performed using whole section slides. Tissue cores from formalin fixed paraffin embedded donor blocks (2 cores per block) were arrayed to create a TMA of cores measuring 2.0 mm each. The TMAs were stained for MLH1, MSH2, PMS2 and MSH6 and results were compared to the findings in the whole sections.

Results: Of the 672 cores stained for the MMR proteins, there was insufficient tumor for analysis of stains in 3 cores (3/672=0.4%). Of the remaining 669 cores, 4 showed discrepant results on the TMA with their respective whole sections. One showed an absence of PMS2 on the whole section, and its presence on the TMA; while a second showed the presence of MLH1 on the whole section, but its absence on the TMA. A third tumor that had previously showed the presence of all 4 MMR proteins in the whole section showed absent MSH2 and MSH6 on the TMA. Upon review of the whole section, we found the tumor had a heterogeneous staining pattern, and that the core had been taken from the area of the tumor where MSH2 and MSH6 were negative. The overall concordance for staining TMAs was 99.4% (665/669). Since 20 tumors were evaluated on each TMA, the cost for performing IHC was 1/20th the cost for staining the whole section slides.

Conclusions: There is a high concordance rate between TMAs and whole sections when stained for the MMR proteins. We found discrepant results in less than 1% of our cases. A heterogeneous staining pattern is a potential pitfall of TMA analysis, but a rare finding. The simultaneous staining of 20 tumors using a TMA results in up to a 20-fold decrease in cost compared to using conventional whole sections while maintaining an overall concordance rate of over 99%.

476 Histological Charactestics Predictive of Nodal Metastasis in T1 Colorectal Carcinoma

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Background: Lymph node evaluation is an important prognostic factor in colorectal cancer. With advances in endoscopic skills and instruments, increasing numbers of T1 colorectal carcinomas are being detected. It is widely accepted that once the cancer cells have invaded into the sub mucosal layer, they can metastasize to the regional lymph nodes or even distally to the liver or other organs. Despite recent advances in the knowledge of various clinical, biologic and pathologic features that relate to the prognosis of colorectal carcinoma, the risk of lymph node metastasis in patients with colorectal carcinoma invading the sub mucosal layer is still undetermined. The purpose of this study was to evaluate the frequency of lymph node metastasis in patients with T1 colorectal carcinoma and to determine whether any histologic features are predictive of nodal metastasis.

Design: A retrospective review of all colorectal resection performed at the Saint Barnabas Medical Center, Livingston, New Jersey, for colorectal cancer from January 1st 2000 through December 31 2005. 37 cases with AJCC 6th ed. T1 stage were identified. Of these 37 cases, positive lymph nodes were identified in 6 cases. 6/37 (16%). Demographic and pathological data (age, sex, size of tumor, site of tumor, histological type, lymphovascular space involvement, extra cellular mucin production) were evaluated.

Results: Of 680 patients who had colon resections in the study period, 37 cases had T1 cancers. The incidence of T1 lesion is 5.4%. 6 cases had positive lymph nodes. The frequency of lymph node metastasis is 16%. 33% were from the right side while 67% were from the left side. The mean tumor size was 2.6cm. The mean number of lymph nodes was 14. 30% of tumors were well-differentiated (low grade) histological type, while 48% were of moderately differentiated type and 22% were of poorly differentiated type. In the node positive cases, 1(16%)was well differentiated, 1 (16%) was moderately differentiated and 4(67%) were poorly differentiated. One case each from node positive and node negative category, showed lymphovascular invasion. There was a trend towards greater extracellular mucin production in node positive cases.

Conclusions: The incidence of lymph node metastases in T1 colorectal carcinoma is 16%. Lymphovascular invasion is not highly correlated with the risk of nodal metastasis, while the presence of any extracellular mucin showed a trend towards increasing the likelihood of nodal metastasis. These findings may be helpful in histologic evaluation of limited (mucosal) resection of early lesions.

477 Poorly Differentiated Endocrine Carcinomas of the Pancreas: A Clinicopathologic Analysis of 9 Cases with Emphasis on the Large Cell Endocrine Carcinoma Variant

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Background: Poorly differentiated endocrine carcinomas of the pancreas (PDECP) are rare.The large cell variant, better characterized in the lung, is less well understood when it occurs as a primary pancreatic tumor.We describe our experience with this tumor.

Design: Retrospective review of pathology and clinical records of patients with a diagnosis of PDECP in our database (1990-2006). On review, we used the WHO classification of lung tumors to categorize the PDECP into small cell (SCC) and large cell (LCEC) categories.

Results: Nine cases met criteria for PDECP. These constituted about 3% of the pancreatic endocrine neoplasms seen in our department. Of the 9 cases, 8 met criteria for LCEC and 1 for SCC. The mean age (3 males and 6 females) was 62 years. Three patients had a remote history of smoking.Symptoms were dyspepsia (4 pts) and weight loss (2 pts). Mean tumor size was 5.0 cm. Equal numbers of tumors were located in the head and tail region, while one was located in the body. Grossly, most tumors were tan and well-circumscribed. Of the 7 patients in whom lymph nodes were available for review, 5 had nodal metastases.Two patients had metastatic liver disease. All patients were treated with resection except one with superior mesenteric artery involvement. The LCECs showed endocrine arcitectural patterns (nesting, palisading , pseudoglandular), abundant necrosis and a high mitotic rate (mean 35/10 hpf). The cytologic features were large cell size, vesicular or fine chromatin and/or frequent nucleoli. Vascular invasion was seen in 85% and perineural invasion in 60% of the cases. One of the cases had an associated low-grade pancreatic endocrine neoplasm and one had a ductal adenocarcinoma .All of the HGNECs were positive for chromogranin or synaptophysin. Trypsin and chymotrypsin were negative. TTF-1 was done in 3 cases, 1 of which was positive ; however, a thorough workup failed to reveal any other primary site. Follow-up was available for 6 patients: 5 developed liver metastases, 1 had recurrent pancreatic disease and 1 had lung metastasis. Overall, 44% died of disease (mean follow-up,14.0 months). Of those still alive (mean follow-up 18 months) 2 had no evidence of disease, 1 had liver metastasis and no information was available in 2 patients.

Conclusions: PDECs of the pancreas are rare. Due to their rarity, metastasis especially from the lung and acinar differentiation should be ruled out before making the diagnosis. The clinical course of PDEC, including LCEC, is highly aggressive.

478 Differential Gene Expression Profiling of MGMT Deficient Colorectal Cancer Reveals a Carcinogenic Pathway Distinct from Other Mismatch Repair Intact Tumors

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Background: O^6-methylguanine-DNA methyyltransferase (MGMT) removes cytotoxic O^6-alkyl adducts from normal cells and protects tumor cells from alkylating agents. Loss of MGMT expression by promoter methylation is common in colorectal cancer (CRC), yet MGMT deficient CRC does not share the clinicopathologic features of other methylated phenotypes such as mismatch repair (MMR) deficient tumors, suggesting differences in etiology and pathogenesis. We examined the gene expression profiles of MGMT deficient and MGMT/MMR intact (stable) CRCs to identify those genes differentially expressed.

Design: RNA was extracted from 10 MGMT deficent tumors and 10 MGMT/MMR intact tumors containing greater than 70% tumor cellularity and hybridized onto Affymetrix U133 plus 2.0 arrays. Probe intensities were normalized using the GCRMA method. By supervised analysis using the rank-product method, differentially expressed genes were identified by requiring a geometric-mean fold-change of 2.0 and a false discovery rate <0.4% for up-regulated genes and <0.2% for down-regulated genes. We used the program GeneSifter to identify the significant KEGG pathways and GO catagories that were over-represented in the list of differentially expressed genes.

Results: We identified 343 genes genes that were differentially expressed (133 up, 210 down) in MGMT deficient CRCs. Significant underexpression occurred in genes involved in cell adhesion, cell communication and maintenance of the cytoskeleton such as *MUC2*, *SLC26A3*, *KER20*, *MMP3* and *CEACAM7*. Significant overexpression occurred in genes involved in the cell cycle and cellular metabolism such as *LRP4*, *CA9*, *GRM8*, *ACSL6* and *H2AFJ*.

Conclusions: Out of the 47,000 transcripts and variants, including 38,500 well-characterized human genes present on the arrays, we identified 343 (0.73%) genes and transcripts that were differentially expressed at a mean fold-change of 2.0 and a false discovery rate <0.4% for up-regulated genes and <0.2% for down-regulated genes. Many of the genes significantly down-regulated in MGMT deficient CRC have biologic functions important for cell adhesion, cell communication and maintenance of the cytoskeleton, while many genes significantly up-regulated are important for cell cycle control and cellular metabolism. The differential expression profiles between MGMT deficient and MGMT/MMR intact CRCs provides support that MGMT deficient CRC is a distinct subset of MMR intact CRC.

479 Lymphatic Permeation Demonstrated in Minimally Invasive Colonic Adenocarcinomas

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Background: The level of submucosal invasion influences the prognosis of patients with colorectal adenocarcinoma. According to the levels of submucosal invasion proposed by Haggitt et al. in 1985 (Haggitt's levels), few colorectal carcinomas limited to Levels 0-3 metastasize to the lymph nodes. However, we experienced three cases of colonic polypoid adenocarcinoma of Haggitt's levels 0-2, which nevertheless showed lymphatic permeation and/or lymph node metastasis.

Design: Three cases of polypoid adenocarcinoma of the sigmoid colon, which were endoscopically or surgically removed all from male patients in their 6th and 7th decades of life, were selected and subjected to examination. Histological assessment with haematoxylin-eosin (HE) and elastica van Gieson, and immunohistochemistry for D2-40, alpha-smooth muscle actin (SMA), and desmin were performed for each lesion.

Results: The gross shape, size, and Haggitt's levels of the three cases were as follows: Case 1: pedunculated polyp, 3×2.8×1.8 cm, Level 2; Case 2: semipedunculated polyp, 0.8×0.7×0.5 cm, Level 0; Case 3: pedunculated polyp, 2×1.8×1.5 cm, Level 1. Case 1 was surgically resected with regional lymph node dissection, and Cases 2 and 3 were removed by endoscopic mucosal resection (EMR). All were gland-forming adenocarcinomas with some sprouting of small clusters of carcinoma cells or single carcinoma cells, and mucinous degeneration. Lymphatic permeation was suggested on

HE preparation in all cases, and confirmed by D2-40 immunohistochemistry in Cases 2 and 3 (not definite in Case 1). All cases were associated with proliferation of SMA-positive, desmin-negative fibroblastic cells (desmoplastic reaction) associated with an increased number of D2-40 positive lymphatic vessels, in particular even reaching above the muscularis mucosa in Case 2. Regional lymph node metastasis was present in Case 1 (three positive nodes out of 14 dissected nodes), but not in Case 2 after post-EMR additional surgical resection.
Conclusions: Our limited case study demonstrates that colonic adenocarcinomas permeate lymphatic vessels even when they are limited above the muscularis mucosa (Haggitt's level 0). Aside from this, lymph node metastasis occurs even when the level of invasion is limited to low Haggitt's levels. These findings seem to be associated with remodeling of the stromal components with proliferation of lymphatic vessels in the invasive area. Careful examination and clinical observation are needed, even if colonic carcinomas are minimally invasive.

480 Identification of Novel CpG Methylation Markers of Colonic Adenomas and Adenocarcinomas

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Background: CpG island methylation is an epigenetic mechanism of gene inactivation in colorectal cancers. We recently identified three novel epigenetic markers of colorectal neoplasia, including the v-raf-1 murine leukemia viral oncogene homolog (RAF1), plasma glutamate carboxypeptidase (PGCP), and the transforming growth factor beta receptor III (TGFBR3). The aims were to evaluate the CpG island methylation status of these genes in sporadic colonic adenomas and adenocarcinomas, non-neoplastic mucosa of the same patients and of patients without colonic neoplasms.
Design: We studied 37 colon neoplasms (21 adenocarcinomas and 16 adenomas) and normal background mucosa. Normal colonic mucosa of 10 patients without colonic neoplasms was used as control. The mean ages of patients with adenomas, carcinomas or controls were 67, 64, and 53 years, respectively. The methylation status of CpG islands of TGFBR3, RAF1 and PGCP genes was evaluated by methylation specific PCR (MSP) of bisulfite modified DNA.
Results: TGFBR3 and RAF1 were more frequently methylated in carcinomas and adenomas as compared to normal colonic mucosa of patients without colonic neoplasms ($P<0.001$). Methylation of TGFBR3, RAF1 and PGCP was detected in most adenomas and carcinomas, whereas it was less frequent in the non-neoplastic colonic mucosa of the same patients (Table).
Conclusions: The TGFBR3, RAF1 and PGCP 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 TGFBR3, RAF1 and PGCP in colonic carcinogenesis. Epigenetic regulation of these genes occurs early in colonic neoplasia since they were similarly methylated in adenomas and carcinomas. CpG methylation testing of these genes may be diagnostically useful for detection of colonic neoplasms.

	NC	NA	NT	A	T	P Value	P Value
Number of Cases	10	16	18	16	21	NA vs A	NT vs T
TGFBR3 (%)	0	13	10	88	90	p<0.001	p<0.001
RAF1 (%)	20	19	24	100	86	p<0.001	p<0.001
PGCP (%)	40	19	10	75	67	p=0.004	p<0.001

Table: Frequency of positive CpG methylation status of TGFBR3, RAF1 and PGCP in NC: Normal colonic mucosa from patients without neoplastic disease. NA: normal colonic mucosa adjacent to colonic adenoma. NT: Normal colonic mucosa adjacent to colonic carcinoma. A: colonic adenoma. T: colonic carcinoma.

481 The Assessment of Specimens Procured by Endoscopic Ampullectomy

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Background: In the past decade, endoscopic mucosal resection of ampullary adenomas has been increasingly utilized. Few studies, however, have assessed the specimens procured by this method as well as the procedure's utility. This study reviews our experience with specimens obtained by endoscopic ampullectomy.
Design: A cohort of patients undergoing endoscopic ampullectomy for presumed ampullary adenomas was identified (2000-present). Ampullary biopsy diagnoses before and after endoscopic ampullectomy were recorded. Ampullectomy specimens were assessed for the following histologic features: diagnosis, histologic subtype, presence of high grade dysplasia (HGD), involvement of underlying ampullary glands/ducts by adenoma, specimen integrity, and margin status. Previous biopsy diagnoses were compared to the results of ampullectomy, and the Fisher's exact test was used to correlate histologic features with disease persistence.
Results: Forty-six patients underwent endoscopic ampullectomy for presumed adenoma (19 females and 27 males, ages 25-88). For the 23 patients with previous biopsy, there was diagnostic agreement for 65%. The histologic features of the 37 cases with follow-up were as follows: diagnosis (negative 2, reactive 6, tubular adenoma 11, tubulovillous adenoma 12, carcinoma 5, other 1), differentiation (intestinal 24, mixed intestinal-pancreatobiliary 2, unclassified 2), HGD 1, ampullary gland/duct involvement 17, specimen integrity (intact 18, fragmented 19), margin status (positive 16, negative 2, could not be assessed 11). Extensive thermal artifact was common with these specimens. 70% of patients had documented persistent ampullary neoplasia. No histologic feature reviewed had a statistically significant relationship to disease persistence.
Conclusions: Although some patients who undergo ampullectomy will be found to have only reactive changes, a significant proportion of these patients will ultimately be found to have persistent disease. The assessment of margin status with these specimens is often hindered by fragmentation and thermal artifact. Predicting persistent disease is not possible based on histologic assessment. Overall, endoscopic ampullectomy does not appear to represent a definitive therapeutic option and should be coupled with careful endoscopic follow-up.

482 Growth Pattern-Based Method of Colon Cancer Grading: A Novel Alternative To Estimating Tumor "Differentiation"

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Background: The currently used system of grading colon cancer (CC), which is based on the percentage of gland formation, has limitations, such as poor interobserver reproducibility, which probably contributes to the lack of correlation with outcome in some studies. For instance, the majority of CC are classified as low grade (moderate or well-differentiated), but there is great variation in the outcome of patients with these tumors. Low-grade tumors encompass a wide spectrum of morphologic growth patterns in CC. The aim of this study was to evaluate the utility of a novel growth pattern-based method of CC classification as a better alternative to grading tumors based on percentage gland formation.
Design: Routinely processed tissue sections from 145 non-mucinous consecutive CCs were reviewed by 3 GI pathologists who identified 6 overall morphologic patterns of tumor growth. These were designated as type-1: lobular, type-2: papillary/serrated, type-3: adenomatous, type-4: cribriform, type-5: tubulo-glandular, and type-6: scirrhous, based on the predominant growth pattern in the tumor. The tumor growth pattern was correlated with the clinical and pathologic features, such as age, sex, site, size, presence or absence of LVI, depth of invasion, lymph node metastases and distant metastases, among others.
Results: The type of growth pattern did not correlate with demographic characteristics, such as patient age or gender, nor with tumor location or size (all $p>0.05$). However, significant associations ($p<0.05$) were noted between certain patterns of growth (ex. Type 4, 5 and 6) and depth of invasion, lymph node metastases, distant metastases, and lymphovascular invasion. Segregating similarly "differentiated" tumors (ex. moderately differentiated) into different growth patterns significantly increased the correlation with several pathologic indicators of tumor aggression, including pathologic stage. The addition of non-predominant (secondary) patterns of growth in the analyses did not improve the overall predictive value.
Conclusions: Our novel growth pattern-based method of CC grading may provide additional or more useful clinical predictive information compared to traditional tumor grading based on percentage of gland formation. Correlation of growth pattern with survival should be performed in future multivariate studies.

483 Human Papillomavirus (HPV) Detected by PCR Is Common in Colon Tissues but Is Unlikely To Be a Significant Factor in Colon Cancer Etiology

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Background: Human papillomavirus (HPV) is recognized as a necessary cause of cervical carcinoma. There is evidence that HPV may also play a role in the etiology of other tumors including breast, esophageal, head and neck, and ovarian carcinomas; additionally, recent studies have identified HPV in 51% up to 74% of colon carcinomas indicating HPV might impact these tumors too.
Design: Formalin-fixed, paraffin-embedded (FFPE) colon (ascending, descending, and sigmoid) carcinomas and normal adjacent tissue (NAT) samples were obtained for 34 recent patients. In addition, 15 FFPE normal colon tissue samples from patients treated for constipation, diverticulitis, or ulcerative colitis were collected. Cervical carcinoma samples were used as positive controls. Sections of FFPE retail beef (cut between colon samples during tissue preparation for DNA extraction) were used as negative controls. DNA extracts were tested for HPV using high (H) and low (L) sensitivity modifications of the GP5+/6+ PCR assay. HPV type was determined by dot blot hybridization. Biotinyl-tyramide based chromogenic *in situ* hybridization (CISH) was performed on selected samples that tested HPV positive with both PCR tests.
Results: Sixteen of 34 (47%) colon carcinomas and 8 (23.5%) associated NAT tested HPV positive (types 16, 18, 35 or 40) following HGP5+/6+ assay (P=0.075); 5 HPV negative colon carcinomas had associated NAT that tested HPV positive. Following LGP5+/6+ assay, 6/34 (17.6%) colon carcinomas and 3 (8.8%) NAT tested HPV positive (types 16, 18, or 35) (P=0.476); 1 HPV negative colon carcinoma had HPV positive NAT. Of 15 normal colon samples from patients without neoplastic disease, 7 (46.7%) tested HPV positive (types 16 or 18) after HGP5+/6+ PCR, and 3 (20%) tested positive after LGP5+/6+ PCR. There was no significant difference in the prevalence of HPV in cancer tissues compared to normal tissues from non-cancer patients (P=1.000, H or LGP5+/6+). HPV was not detectable by CISH in PCR HPV positive colon carcinomas, whereas HPV was readily detectable in cervical carcinomas.
Conclusions: These data support recent findings that HPV is common among colon carcinomas (dependent upon the PCR assay). However, our data showed no significant difference in the prevalence of HPV between tumor and normal tissues; this, together with the non-detection of HPV by CISH, implying HPV is present in low-copy number, suggests HPV is unlikely to have causal significance for colon cancer.

484 Anal Intraepithelial Neoplasia (AIN): Correlation of Grade with p16INK4a Immunohistochemistry (IHC) and HPV In Situ Hybridization (ISH)

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Background: Accurate diagnosis and grading of AIN can be problematic, especially in separating AIN from anal transitional-zone epithelium. IHC for $p16^{INK4a}$ correlates with HPV integration into the host genome, and HPV subtyping by ISH for low and high-risk subtypes is now readily available. To investigate if $p16^{INK4a}$ would help in more accurately diagnosing and grading AIN, we separately assessed these stains in a "blinded" manner on a large number of consecutive anal biopsies and anal tissues and correlated the findings with the diagnosis and grade of AIN.

Design: 133 consecutive anal tissue specimens, from 128 patients were studied. 108 were anal biopsies and 25 were hemorroidectomy specimens. All specimens were stained with hematoxylin and eosin (H&E), p16^{INK4a} (Lab Vision), and HPV ISH (Ventana HPV-III Inform™). AIN grade (1 thru 3) was correlated with nuclear intensity of p16^{INK4a} IHC (0-3+). Agreement between tests was tested for statistical significance using the kappa value (k).
Results: 100% of AIN 2 and 3 were positive for p16^{INK4a}; whereas 80% of AIN 1 were positive (k value was 0.61 [substantial agreement]). 79% and 9% of AIN 3 were positive for high-risk (HR) and low risk (LR) HPV, respectively; whereas, 75% and 5% of AIN 2 were positive for HR and LR HPV; and 40% and 24% of AIN 1 were positive for HR and LR HPV (k value was 0.62 [substantial agreement]). 100% of HR HPV were positive for p16^{INK4a} and 85% of LR HPV were positive for p16^{INK4a}. Only 6% of negative p16^{INK4a} were positive for HPV, and all were LR; yet, 43% of negative HPV showed p16^{INK4a}. Of the latter, 40% were 3+ by p16^{INK4a} (k value was 0.56 [moderate agreement]). Finally, 30 cases were negative by H&E and p16 staining. Of these, 2 (7%) were positive for HPV (both = LR subtype). 3 cases positive for HR HPV were negative by H&E with only patchy p16 positivity.
Conclusions: AIN (all grades) correlated substantially with expression of p16^{INK4a} and HPV, especially HR HPV subtypes. Likewise, HPV correlated with p16^{INK4a}, but slightly less so than for AIN. Thus, IHC for p16^{INK4a} is very useful in distinguishing true AIN from benign mimics, such as benign transitional-zone epithelium. The diagnostic utility of IHC for p16^{INK4a} is greatest in AIN 2 and 3.

485 Histologic Features That Distinguish Sessile Serrated Adenomas and Hyperplastic Polyps Are Related to Polyp Size: Smaller Polyps Merge into a Single Morphologic Entity

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Background: Large sessile serrated adenomas (SSAs) and hyperplastic polyps (HPs) are morphologically distinctive. Small polyps often lack these distinctive features and are often difficult to classify. The goal of this study was to corollate polyp size with histologic features of serrated polyps to begin to establish diagnostic criteria to separate small SSAs and HPs.
Design: 923 SSAs and HPs were retrieved from our file and grouped by mm size (2mm, 3mm, 4mm, 5mm, 6mm, 7mm, 8mm; N=60, 212, 215,196,148, 69, 23, respectively). An equal or similar number of polyps were from the right vs left colon for each mm group. The number of normal, mildly-, and moderately (mod)-dilated, and flask-shaped crypts was recorded. For each crypt shape, the number with basilar, mid and superficial upwards extension of immature cells, mitoses, serration, and type of cytoplasmic differentiation was recorded. Polyps were blindly re-reviewed at a later date and diagnosed as SSA, HP, Indeterminate (Ind).
Results: The percentages of normal crypt bases/ polyp were 84% and 24% in 2 mm and 8 mm polyps, respectively, whereas the percentage of crypts with mod-dilated or flask shaped bases increased from 2% and 0% respectively in 2 mm polyps to 41% and 18% in 8 mm polyps. The percentage of polyp crypts with mid-level immature cells and mitoses was <10% in all 2 and 3 mm and most 4 mm polyps to >50% in >5 mm polyps. There was uniform cytoplasmic maturation in all 2 and 3 mm, whereas almost all >5mm polyps had small clusters of crypts with similar cytoplasmic maturational features that were different from the features of adjacent crypts. The percentage of 2mm, 3mm, 4mm, and 5mm polyps diagnosed as HP, Ind, SSA were (91%,9%,0), (57%,41%,2%), (29%,49%,22%), and (13%,24%, 63%), respectively.
Conclusions: There was progressive increase in the extent of changes with increasing polyp size beginning with basilar crypt dilation, followed by upwards expansion of immature epithelial cells and mitoses, and ending with flask-shaped serrated crypt bases and full-thickness immature epithelial cells. Small polyps were singularly HP-like. 50% of 4 mm polyps had indeterminate features, whereas whereas 63% of 5mm polyps had predominantly SSA features. Polyp size may be an marker of the extent of underlying molecular alterations. SSAs and HPs may be related entities and derive from a common pool of lesions. SSAs emerge from this pool due to greater levels of cell cycle control slippage and cell signaling pathway perturbations.

486 hRTVP-1 Is Significantly Downregulated in Esophageal Adenocarcinoma

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Background: The human RTVP-1 (hRTVP-1) gene is involved in p53-mediated apoptosis and cell cycle regulation. Hypermethylation of the hRTVP-1 gene leads to its inactivation, and to decreased or absent protein expression. The aim of this work was to determine whether hRTVP-1 expression is downregulated in esophageal adenocarcinoma.
Design: Sections from esohagectomy specimens including 13 with Barrett's metaplasia negative for dysplasia (BMND) and 22 adenocarcinomas (ACA) were stained for the hRTVP-1 protein using the immunoperoxidase method. The percentage of positive cells was scored on a scale of 0-5 with 0 completely negative; 1, 1-10%; 2, 11-25%; 3, 26-50%; 4, 51-75%; and 5, >75% of the cells positive. The intensity of staining was scored as 0, completely negative; 1, weak; 2, moderate; and 3, strong intensity.
Results: hRTVP-1 staining was both nuclear and cytoplasmic, and was completely abolished by preincubating the antibody with the immunizing peptide. The immunostaining score (IC) score for the BMND cases ranged from 6-15, whereas the score for ACA ranged from 0-10 with 4 of the ACA cases completely negative and 14 having very low IC scores (0-2). The mean IC score for the ACA, 3.3, was significantly lower than that for BMND,11, ($p<0.0001$, unpaired t-test). In a smaller subset of the cases in which both BMND and ACA were from the same esophagectomy specimens, the mean IC score for ACA (4.5) and BMND (10.9) were also significantly different ($p=0.0105$, paired t-test).
Conclusions: We conclude that the majority of esophageal adenocarcinomas have significant reduction of hRTVP-1 protein expression suggesting that hRTVP-1 hypermethylation may play a significant role in malignant transformation in Barrett's esophagus.

487 Identification of Hepatocyte Paraffin 1 (Hep Par 1) Antigen

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Background: Hepatocyte paraffin 1 (Hep Par 1, OCH1E5.2.10 clone), a murine monoclonal antibody, is widely used in surgical pathology practice to determine the hepatocellular origin of neoplasms. This antibody is specific for well-differentiated normal and neoplastic hepatocytes. The immunohistochemical staining pattern on formalin-fixed, paraffin-embedded tissues suggests localization of the antigen to the membrane of a cytoplasmic organelle, though the exact antigen has never been determined. The aim of this study is to identify and characterize the Hep Par 1 antigen from normal and neoplastic human liver tissue by biochemical and immunological approaches.
Design: To determine the identification of the antigen, Western blot analysis was utilized, with the Hep Par 1 antibody (OCH1E5 clone, Dako), to detect the presence of the protein within normal and neoplastic liver tissue. Human hepatoma cell line (Huh7) and a Chinese hamster cell line (CHO) were used as negative controls. Immunoprecipitation techniques were used to isolate the protein; mass spectrometry was used to identify the protein.
Results: Immunoprecipitation and western blot analysis identified a distinct protein band, approximately 165 kD, within both normal (7/7) and neoplastic liver tissue (4/4). The protein band was not present within Huh7 and CHO cell lines (negative controls) or several other non-liver tissues. The protein was purified and analyzed by mass spectrometry, and was identified as carbamyl phosphate synthetase I (CPS1). The Hep Par 1 antibody-immunoprecipitation complex was analyzed by Western blot analysis using a rabbit anti-CPS1 antibody, confirming the results of mass spectrometric analysis.
Conclusions: The Hep Par 1 antibody binds to a large protein present within both normal and neoplastic liver cells. This protein was identified as carbamyl phosphate synthetase I, a liver specific intramitochondrial urea cycle enzyme. Poorly-differentiated liver cancer cells do not express this protein, suggesting it is only expressed in well-differentiated hepatocytes. Further examination of carbamyl phosphate synthetase I protein expression in hepatocytes may benefit studies on liver cell differentiation.

488 ProEx C Is a Marker for HPV-Associated Anal Lesions

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Background: ProEx C (Tripath Imaging, Burlington, NC) is a recently developed marker composed of antibodies to minichromosome maintenance protein 2 (MCM2) and topoisomerase IIA, two cell cycle proteins shown by DNA microarray analysis to be associated with cervical neoplasia. More recent reports suggest that ProEx C may be useful in the diagnosis of high grade squamous intraepithelial lesions in cervical cytology. Anal epithelium, like cervical epithelium, has a proclivity for HPV-infection and their HPV-associated intraepithelial lesions share many similarities. This study was designed to assess ProEx C immunostaining in anal epithelium so as to determine if it is useful as an adjunct in evaluation of biopsies for diagnosis and grading of anal intraepithelial neoplasia (AIN).
Design: Slides of 85 anal biopsies were retrieved from our files. Patients (77 male; 8 female) ranged in age from 19 to 72 years (mean: 41 yrs). Based on H&E stains, diagnoses were: negative (27), condyloma without overt dysplasia (9), AIN I (12), AIN II (24), and AIN III (13). Slides of each biopsy and appropriate controls were immunostained for p16 (Biocare Medical), Ki67 (Ventana), and ProEx C. As previously reported by us, p16 was recorded as negative, spotty, or band-like (>90 % of lesional cells stain) and Ki67 was recorded as negative or positive (>50% of lesional cell nuclei stain). ProEx C was recorded as negative or positive (band-like staining above the basal third of the lesional epithelium). In 35 (41%) cases (5 negative, 2 condyloma, 7 AIN I, 13 AIN II, 8 AIN III), ISH for high risk HPV was also performed utilizing the DAKO-GenPoint HPV DNA probe. ProEx C staining was correlated with diagnoses and p16/Ki67 stains.
Results: ProEx C positivity was seen in 30% negative, 78% condyloma, 50% AIN I, 67% AIN II, and 85% AIN III cases. ProEx C staining correlated with HPV infection (if all lesions are considered, $p=0.0009$; if only HR HPV ISH+ biopsies are considered, $p=0.03$). Although the percentage of cases positive for ProEx C increased from AIN I to AIN III, statistical significance to distinguish high from low grade lesions was not achieved. Strong correlation was detected with p16 positivity (spotty >10% or band) and Ki67 positivity ($p=0.004$ and $p=0.0002$, respectively).
Conclusions: Our findings suggest that in anal epithelium band-like staining for ProEx C above the basal third (a) is strongly associated with HPV infection; (b) correlates with p16/Ki67 staining; (c) does not distinguish high from low grade AIN.

489 Prevalence and Clinical Significance of Serrated Lesions in Ulcerative Colitis

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Background: Patients with long standing ulcerative colitis (UC) are at increased risk for colorectal adenocarcinoma. Most adenocarcinomas in this setting arise from flat or polypoid "tubular" dysplasia. Serrated lesions occur in patients with UC, but they have not been well-characterized with regard to their association with carcinoma. We describe the clinicopathologic features of serrated lesions in colons resected for UC-associated adenocarcinoma, and compare them to findings in colons resected for medically refractory UC.

Design: We reviewed the surgical pathology files for patients with adenocarcinoma complicating UC. Biopsy-proven UC for at least 10 years was a requirement for inclusion in the study. 114 patients who met this criterion had resection between 1984 and 2006. 114 age- and sex-matched patients with biopsy-proven UC who had resection for other indications and who were reportedly negative for dysplasia served as a control group. We reviewed all slides (mean 16 per case, range 8-35) and noted the presence of hyperplastic polyp (HPP), sessile serrated polyp (SSP) and serrated adenoma (SA), and the physical relationship of said lesions to adenocarcinoma. Immunohistochemical stain for MLH1 was applied to 91 (80%) of the adenocarcinomas.
Results: Of 114 colectomies with adenocarcinoma, 28 (25%) had at least one serrated lesion. Seventeen (15%) had serrated changes adjacent to adenocarcinoma; 12 of these were classified as SA and the remainder as serrated changes without dysplasia. Twenty-one (18%) had serrated lesions not contiguous to adenocarcinoma; these comprised SA (10), SSP (4) and HPP (7). Ten patients had serrated lesions both contiguous to tumor and distant from it. Among the 91 adenocarcinomas tested, 3 (3%) showed loss of staining for MLH1. The 3 MLH1 negative cancers included 1 mucinous adenocarcinoma, 1 medullary carcinoma and 1 typical adenocarcinoma. None of the 3 MLH1-negative tumors was adjacent to a serrated lesion. In contrast, of the 114 colectomies performed for medically refractory disease and lacking adenocarcinoma, only 4 (3.5%) had serrated lesions and all of these were HPP; none had SSP or SA.
Conclusions: Serrated lesions are seen in a significant minority of patients with UC complicated by adenocarcinoma, but are rare in colectomies for medically refractory disease. Based on the low prevalence of MLH1 loss (3% of cancers) and the absence of serrated lesions associated with MLH1-negative tumors, we suggest that some adenomas arising in the setting of ulcerative colitis arise via the CpG-island methylator pathway.

490 Immunohistochemical Characterization of Serrated Lesions in Inflammatory Bowel Disease

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Background: Long-standing idiopathic inflammatory bowel disease (IBD) is an important risk factor for colorectal neoplasia. Polypoid or flat dysplasias are considered the traditional precursors to IBD-associated carcinomas. Serrated lesions have been described occasionally in IBD, but little is known about their neoplastic potential and immunophenotype.
Design: We searched the computerized Surgical Pathology files from 1996-2006 for cases containing the terms "colitis" and "serrated" or "hyperplastic," eventually yielding 53 serrated lesions among 13 patients with IBD. We classified the lesions using published criteria (*Am J Clin Pathol* 2005;124:380-91) as hyperplastic polyps (HPP; n=18), incidental serrated change (not seen endoscopically as a polyp; n=12), serrated adenoma (SA; n=10), sessile serrated adenoma (SSA; n=8), mixed polyps (n=4) and serrated carcinoma arising from serrated adenoma (n=1). Usual ("tubular") dysplastic lesions (n=12) were also studied when present. All lesions were subjected to immunohistochemistry for p53, p16, β-catenin, MLH1, MSH2, MSH6 and PMS2. In all but 2 lesions, nondysplastic mucosa was present as a control.
Results: Twelve patients had ulcerative colitis and 1 Crohn's disease. Mean age was 55 yrs (range 20-82 yrs), with 8M and 4F. Of cases with satisfactory immunostaining, p16 expression was seen in 17/18 (94%) HPP (mean 21% labeling), 6/10 (60%) incidental serrated change (mean 20% labeling), 9/9 (100%) SA (mean 42% labeling), 7/7 (100%) SSA (mean 45% labeling), 2/3 (67%) mixed polyps (mean 60% labeling), 1/1 (100%) serrated carcinoma (90% labeling) and 9/11 (81%) tubular dysplasias (mean 36% labeling). In contrast, p16 labeling of background mucosa was noted in only 3 of 11 patients (5% of crypts in 1, 2% in 1, rare cells in 1). p53 was negative in all incidental serrated change and HPP, and was overexpressed in 3/10 (30%) SA, 1/8 (12%) SSA, 2/4 (50%) mixed polyps and 7/12 (58%) tubular dysplasias. Nuclear labeling for β-catenin was seen in only 3 dysplastic lesions (1 SA, 1 mixed polyp, 1 tubular adenoma). There was no loss of MLH1, MSH2, MSH6 or PMS2 in any IBD-associated lesions.
Conclusions: p16 is aberrantly expressed in the majority of serrated lesions (including nondysplastic serrated epithelium) in IBD as well as in conventional "tubular" dysplasias, suggesting that it may be an early event in epithelial hyperplasia. In contrast, both p53 overexpression and nuclear accumulation of β-catenin are largely confined to dysplastic lesions, whether serrated or tubular. MSI does not appear to play a significant role in precancerous lesions of IBD.

491 Long Term Histopathologic Features of the Colorectal Mucosa Following *Clostridium difficile* Colitis

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Background: Bacterial enterocolitides (e.g. due to Salmonella and Shigella) may be associated with persistent histologic changes mimicing those of idiopathic inflammatory bowel disease (IBD). Although the features of acute *Clostridium difficile* colitis (CDC) are well known, the long term histologic changes following CDC have not been well characterized.
Design: All patients with a positive *C. difficile* stool antigen assay from 2000-2004 were identified and all colorectal biopsies from those patients reviewed. Exclusion criteria included colorectal biopsies taken before or within 1 month after the positive stool assay as well as patients with established IBD. Histologic findings in biopsy material were correlated to clinical features and follow-up interval for each patient.
Results: Eighty four patients with a positive stool assay and subsequent colorectal biopsies were identified. 114 colorectal biopsies from these patients were reviewed. The mean age was 46 ± 21.5 yrs (range 1-82 years), the M:F ratio was 43:41, and the demographics were 51W:31B:2O. 53 patients (63%) were immunocompromised. Overall, 47 biopsies (41%) showed no mucosal abnormality, 46 (40%) showed acute inflammation and/or reactive changes only, and 21 showed chronic inflammatory changes with or without acute inflammation (18%). Within 3 months of a positive stool assay, 14 (41%) of biopsies showed no histopathologic changes, 3 (17%) showed acute inflammation or reactive changes only, and 17 (50%) showed chronic inflammatory changes, with or without superimposed acute inflammation. Moreover, at 3-8 months, at 8-12 months and at >12 months, 33 to 44% of patients continued to have chronic inflammatory changes in colorectal biopsy material. No relationship was found among immunocompromised status and histologic features in follow-up biopsies.
Conclusions: Similar to that reported for bacterial enterocolitides due to Shigella or Salmonella, antibiotic-associated CDC may result in histopathologic changes that simulate IBD. In our study, up to 33% of patients demonstrated such changes that persisted for at least one year.

492 Expression of Epidermal Growth Factor Receptor and HER2 in Sporadic Primary Small Intestinal Adenocarcinoma

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Background: Primary small intestinal adenocarcinoma (SIA) is rare but tends to present at advanced stages with poor prognosis, making therapeutic management a real challenge. Epidermal growth factor receptor (EGFR) and HER2 are two tyrosine kinase receptors that have served as targeting molecules for specifically designed target-based treatment of a number of malignances such as colorectal adenocarcinoma and breast cancer. Assessment of EGFR and HER2 status in tumor cells has thus become a frequent clinical question. However, the expression of EGFR and HER2 proteins in SIA has not been investigated.
Design: Forty surgically resected sporadic, non-ampullary SIAs (5 duodenum, 22 jejunum, 8 ileum and 5 unspecified) were included in this study. Formalin-fixed paraffin-embedded tissue sections (containing both non-neoplastic small intestinal mucosa and tumor) were immunohistochemically stained for EGFR using a monoclonal antibody (clone 31G7) and HER2 using HercepTest. The staining was interpreted according to Dako EGFR and HER2 phamDx interpretation guide recommended for colorectal and breast carcinomas, respectively. Fluorescence in situ hybridization (FISH) analysis was also performed employing paired commercial SepctrumGreen-labeled centromere enumerating probe 7 and homebrew rhodamine-labeled EGFR DNA probe, or commercially available HER2 FISH pharmDx kit.
Results: No EGFR or HER2 immunoreactivity was detected in non-neoplastic small intestinal mucosa. Positive EGFR staining was observed in 14 cases (35%), among which 12 cases (86%) showed 2+ (moderate) or 3+ (strong) staining intensity and 10 cases (71%) also demonstrated a diffuse staining pattern (>50% of the tumor cells stained). None of the tumors showed HER2 overexpression, although weak staining (1+) was detected in 8 cases (20%), typically involving <5% of the tumor cells. FISH analysis showed none of the 40 tumors to have *EGFR* or *Her2* gene amplification, even in those exhibiting immunohistochemical evidence of EGFR overexpression.
Conclusions: EGFR is overexpressed in one-third of SIAs through mechanisms other than gene amplification. These observations may have important therapeutic implications because EGFR-based therapies have shown promise for other malignancies. However, HER2-based therapies may not be beneficial since this protein is not overexpressed in this group of tumors.

493 High E2F1 Expression Is Related to Worse Outcome in Patients Treated with 5-FU Based Chemotherapy after Curative Resection for Colonic Carcinoma

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Background: To find the most useful method to predict the outcome of 5-FU based chemotherapy in colonic carcinoma, we comparatively analysed TS mRNA expression using real-time RT-PCR, TS gene polymorphism in 5'-untranslated region (5'UTR) and immunohistochemical expression of TS together with E2F1 which is known as a regulator on TS expression.
Design: We used 106 cases of colonic carcinoma treated with 5-FU based chemotherapy after surgical resection. Follow-up duration was 3 to 121 months (73 ±40 months). The analyses were perfomed on normal as well as tumor tissue from each patient. However fresh tissue for real-time RT-PCR were available only for 48 cases among them. TS mRNA expression in normal and tumor tissue, was respectively measured by RotorGene Real-Time Q-PCR system using β-actin as an internal standard and expressed as a TS:β-actin. We also examined tumor to normal ratio of TS:β-actin. For TS gene polymorphism, we performed PCR using DNA from normal and tumor tissue, respectively from 106 patients to detect the copy numbers of 28 bp tandem repeat in 5'UTR (2R/2R, 2R/3R or 3R/3R). We also examined TS and E2F1 immunohistochemical expression using tissue array blocks and scaled them from 1 to 4 for every 25% increase.
Results: TS mRNA expression was 6.76±9.18 in tumor and 3.24±3.28 in normal tissue. The tumor to normal ratio was 4.39±6.48. Suprizingly, mRNA values in normal tissue were variable from 0.11 to 17.39 and significantly correlated with mRNA values of tumor tissue. However TS mRNA was not correlated with survival. The TS genotype of tumor was generally concordant with that of normal tissue. There was no significant difference in survival between genotypes except in stage 3 tumors where patients with 3R/3R TS genotype revealed longer survival than other types. (p=0.0013). On the otherhand, tumors with high E2F1 immunoexpression revealed a significant correlation with poorer survival than tumor with lower expression.
Conclusions: The evaluation of E2F1 expression is more useful than TS expression to predict the outcome in colonic carcinoma treated with 5-FU chemotherapy after surgical resection.

494 Increasing MCM2/TOP2A (ProExC) Expression across the Spectrum of Barrett's Esophagus to High Grade Glandular Dysplasia to Esophageal Adenocarcinoma to Regional Lymph Node Metastasis: An IHC Tissue Microarray Study

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Background: Over 10,000 adenocarcinomas of the esophagus are diagnosed annually in the U.S., and prognosis is poor with 20% overall 5-year survival. Early stage detection can improve survival up to 80%. Minichromosome maintenance 2 (MCM2) and topoisomerase II alpha (TOP2A) levels are known to be upregulated in proliferating cells in association with aberrant S-Phase induction. Herein, we investigate ProEx™C, a novel mouse monoclonal antibody cocktail including both of these markers, in an esophageal tissue microarray to assess expression across the spectrum of esophageal lesions from Barrett's epithelium (BE) to high grade glandular dysplasia (HGGD) to adenocarcinoma (AdCa) to lymph node metastasis (LNM).

Design: Paraffin-embedded archival tissues were harvested from 35 esophageal resections (ENH and AGH 1999-2004) performed for adenocarcinomas arising within 3 cm of the gastroesophageal junction. Patients included 29 (83%) males and 6 (17%) females, mean age 65 years, 9/35 (26%) stage 0/1, 11/35 (31%) stage 2, and 15/35 (43%) stage 3/4. Two tissue microarrays with 3 cores (0.6mm) each of BE, HGGD, AdCa and LNM were created. ProExC (predilute, TriPath) immunohistochemistry (IHC) was conducted with antigen retrieval (decloaking chamber, Biocare Medical) using a mach 2 mouse polymer. Results for ProExC were interpreted by two pathologists (IAC and CDS) and scored with a semiquantitative four-tiered scale.

Results: Strong ProExC IHC reactivity (>50% of cells) was noted in 19% of BE cores versus 89% of HGGD cores (Cochran Mantel Haenszel Chi Square p<0.0011). When a cut of >25% reactivity was used, positive results were recorded as 38% BE, 94% HGGD, 96% AdCa, and 100% LNM.

Conclusions: Our data indicate a statistically significant increase in strong MCM2/TOP2A nuclear expression from BE to HGGD with ProExC showing an 89% sensitivity and a positive predictive value of 84% for HGGD. These findings suggest that ProExC may be a useful marker for esophageal glandular dysplasia in diagnostically challenging cases.

495 Expanding the Spectrum of Sessile Serrated Adenoma

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Background: Sessile serrated adenomas (SSA) are histologically similar to hyperplastic polyps (HP), but biologically comparable to adenomas. SSA are distinguished from HP by 1) proximal location, 2) size >1 cm, and 3) the presence of at least 4 of the following histologic features: i) hyperserrated, ii) dilated, or iii) laterally branched crypts, iv) cytologic atypia or v) mitoses in upper crypts, vi) increased mucin production, and vii) >50% epithelium/stroma ratio. Some small or left-sided polyps may show "SSA-like" features, but their biologic importance and relationships to SSA and HP are unknown. The aim of this study was to evaluate the molecular features of polyps that met histologic criteria for SSA, but were small and/or occurred distal to the right colon.

Design: There were 31 study group polyps (SGP), including 10 spanning 0.5-1 cm and 21 smaller than 0.5 cm, all with at least 4 of the aforementioned histologic features of SSA. The control groups consisted of 6 SSA, as defined above, and 13 HP (all <1 cm). All of the cases were immunostained for MUC2, MUC5AC, MGMT, and Ki-67. A positive result was considered to be staining in >50% of the polyp (MUC2, MUC5AC), loss of staining (MGMT), or presence of staining in the upper crypts (Ki-67). BRAF V600E and K-ras codon 12/13 mutations were analyzed by PCR amplification and direct sequencing.

Results: The SGP were located in the right (3), transverse (9), and left (11) colon, and rectum (8). Nine HP were in the rectum and the rest were from the right (1), transverse (1), and left (2) colon. All of the molecular features of SGP were statistically similar to SSA. All polyps in all groups showed MUC2 expression. MUC5AC staining was present in 16 (52%) SGP and 3 (50%) SSA, but only 2 (15%) HP (p=0.04). Loss of MGMT was seen in 84% of SGP and SSA, compared to 54% of HP (p=0.05). Only a few cases in each group showed increased Ki-67 staining (7 SGP, 2 SSA, and 1 HP). BRAF mutations were present in 20 (65%) SGP, 5 (84%) SSA, and 2 (15%) HP (p=0.003). Only 2 (6%) SGP and 2 (15%) HP had K-ras mutations (p>0.05).

Conclusions: SSA are usually large right-sided lesions that show inactivation of MGMT as well as a gastric-type mucin profile and frequent BRAF mutations. However, smaller, distal colonic polyps may fulfill morphologic criteria for SSA and harbor similar molecular features. Thus, the current diagnostic criteria for SSA may not include some biologically important lesions.

496 Is There Uniformity in the Diagnosis of High Grade Dysplasia in Adenomatous Colon Polyps?

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Background: Adenomatous colon polyps with high grade dysplasia are considered a risk factor for the local recurrence of advanced adenoma and the development of metachronous advanced adenomas and carcinoma. Patients with such adenomas are more closely followed with repeat endoscopy at considerable cost to the medical system. However, the uniformity of the diagnosis of high grade dysplasia has not been studied. In this work we examine the prevalence of high grade dysplasia in a reference set of polyps as interpreted by four independent observers.

Design: 10,780 consecutive polyps removed at a single institution were screened and H&E stained slides of a subset of 393 adenomas were interpreted by three pathologists with expertise in GI pathology from 3 different centers and the IU pathology group. Cochran-Mantel-Haenszel tests were used to compare frequency of interpretation of high grade dysplasia between pathologists.

Results: There was a 10 to 15 fold disparity in the prevalence of high grade dysplasia among the different reviewers that was more pronounced in the group of larger polyps (see table).

Conclusions: The dichotomy of results suggests that there are two different approaches to the diagnosis of high grade dysplasia producing substantial variation in the frequency of this diagnosis. Reproduciblity of shared criteria may also be contributory. The study identifies the need for promulgation of uniform criteria that are evidence based, can be applied reproducibly and promote optimal and cost-effective patient management.

Distribution of histopathologic interpretation of high grade dysplasia in adenomatous colon polyps

Sizes	IU	Pathologist #1	Pathologist #2	Pathologist #3
< 6.0 mm				
(No. of slides)[1]	*(232)*	*(177)*	*(210)*	*(222)*
		(P = 0.0001)	*(P = 0.0004)*	*(P = 0.18)*
Low-grade	98.7%	93.2%	90.5%	97.3%
High-grade	1.3%	6.8%	9.5%	2.7%
6.0 – 9.9 mm				
(No. of slides)	*(161)*	*(133)*	*(159)*	*(158)*
		(P = 0.0001)	*(P = 0.0001)*	*(P = 1.00)*
Low-grade	98.1%	75%	72.3%	98.1%
High-grade	1.9%	24.8%	27.7%	1.9%

[1] The number of slides varied depending on the availability of the slides at the time of interpretation by each pathologist.
P-value reflects the comparison between IU Pathologists and each of the outside pathologists for differences in the distribution of histopathologic interpretations

497 The Prevalence of Solitary Rectal Ulcer Syndrome in Rectal Biopsies

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Background: The pathology of solitary rectal ulcer syndrome (SURS) is well described in the literature. However, in the course of disease, the lesion may appear endoscopically as a polyp without evidence of an ulcer - the so-called polypoid phase of SURS. A recent study suggested that this variant of the entity is poorly recognized by general pathologists (Gastrointestinal Endoscopy 1999; 50: 468-74). The lesion was often mistaken for an adenomatous polyp or chronic inflammatory bowel disease (IBD). In order to judge the significance of this problem, we reviewed rectal biopsies from a general population to determine the prevalence of polypoid SURS.

Design: The computerized records of Indiana University department of pathology were searched for all rectal biopsies taken for polyps, masses, ulcers or nodules from 1997 to 1999. Patients biopsied for frank carcinoma, follow up, or assessment of known IBD, were excluded. The patient records and H&E stained slides were available for review.

Results: 484 rectal biopsies fit the inclusion criteria. Three cases were excluded due to insufficient biopsy material. The vast majority of the biopsies showed either hyperplastic or adenomatous polyps. New diagnoses of IBD were also noted. Out of 481 total cases, 32 (7%) showed the fibromuscular hyperplasia of the colonic lamina propria characteristic of SURS. The patients were 84% female and ranged in age from 23 to 83 years with a mean of 57. The most common presenting symptom was blood in stool (50%). The most common endoscopic appearance was that of a polyp or nodule in 18 (56%) cases. An ulcer was identified in only 11 (34%) cases, the rest were described as "abnormal" or inflamed. The diagnosis was suggested by the clinician in only three cases (9%), all associated with an ulcer.

Conclusions: The study group mirrored the female predominance already noted in the literature. However, the average age of this group of patients was two decades older than that seen in previous studies. The reason for this is unclear but it could be related to the preponderance of polypoid cases rather than the classic ulcer phase. In this cohort of rectal biopsies (excluding frank carcinoma and known IBD), the incidence of SURS was 7%. The polypoid phase of SURS was more than half of these cases or 4% the total. If this group of patients is under-recognized, it could lead to unnecessary treatment or surveillance.

498 Distinct Subtypes of Dendritic Cells, T- and B-Lymphocytes in Primary Eosinophilic Esophagitis: Implications Regarding Pathogenesis and Differential Diagnosis with GERD

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Background: Primary eosinophilic esophagitis (pEoE) and reflux esophagitis (RE) have overlapping histologic features, despite the fact that these entities develop via different pathogenetic mechanisms. To help distinguish pEoE from RE and to further elucidate the pathogenesis of PEoE, a variety of histochemical and immunohistochemical stains, aimed at distinguishing various inflammatory cell subsets in these two disorders, were evaluated, comparing patients with pEoE and RE against normal controls.

Design: Routinely processed esophageal mucosal biopsies from adult (n = 11) and pediatric (n = 33) patients with clinically confirmed pEoE (n = 19), RE (n = 20) and normal esophageal mucosa (n= 5) were stained with antibodies to CD1a, CD3, CD4, CD20, CD123, and with Giemsa to detect Langerhans cells, specific T and B-cell subsets, plasmacytoid dendritic cells and mast cells, respectively. Biopsies were evaluated in a blinded fashion for the distribution and number of cells per high power field (HPF).

Results: A superficial distribution of CD1a cells was seen in pEoE vs RE (75% vs 21%, $p < 0.01$) but not in normal mucosa (0%, $p < 0.01$). CD3-positive and CD-4 positive T-cells were significantly higher in pEoE (50.5/hpf and 13.8/hpf, respectively) versus

RE (34.3/hpf and 6.7/hpf, respectively) and normal mucosa (17.3/hpf and 4.2/hpf, respectively) with $p < 0.01$ for all comparisons. In addition, CD123-positive dendritic cells were more prevalent in pEoE compared to RE (1.03 v 0.2/hpf, $p < 0.05$) and normal mucosa (0.0, $p < 0.01$). No differences in intraepithelial mast cells or CD20-positive B-cells were noted in between pEoE and RE. Mast cells were absent in normal squamous epithelium.

Conclusions: pEoE, in comparison to RE and normal mucosa, is characterized by increased CD3 and CD4 lymphocytes and CD123-positive dendritic cells. This supports a Th-2-type allergic inflammatory response in this disorder and may help differentiate pEoE from RE. Further classification of dendritic cell type Fc-receptors and assessment of CD123-regulatory functions in pEoE may provide additional insight into the pathogenesis of pEoE and potential opportunities for novel treatment methods.

499 Gastrointestinal Tract (GIT) Epithelial Changes Associated with Taxanes: Marker of Drug Toxicity Versus Effect

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Background: Microscopic findings associated with paclitaxel (Taxol®) chemotherapy toxicity were described years ago but whether they are specific for toxicity remains unclear. Further, epithelial changes associated with taxanes can mimic high-grade dysplasia (HGD) in nonneoplastic GIT mucosa. Similar changes associated with colchicine are only seen in patients with toxicity.

Design: GIT specimens were reviewed (221 total; 93 esophageal, 55 gastric cardiac, 48 oxyntic, 7 antral, 8 small bowel, 6 colonic, 3 appendiceal, 1 anal) from 71 patients (pts) (63M, 8F), 38-84 yrs (median, 55 yrs) undergoing chemotherapy for esophageal or lung cancer who had all received taxanes at some time (either paclitaxel [Taxol®] or docetaxel [Taxotere®]). Epithelial changes (mitotic arrest/ring mitoses, apoptosis) associated with taxanes were graded on a scale of 0-3 (0 = no mitotic arrest; 1 = rare arrest; 2 = scattered arrest; 3 = striking mitotic arrest and apoptosis). Samples from the pts taken prior to administration of taxanes were also reviewed; all samples were reviewed without knowledge of the interval between drug doses and the biopsy/resection.

Results:

Findings in Patients Who Received Taxanes

Score	Total Samples	Samples From Patients Who had Yet to Receive Taxanes	Samples with Unknown Date of Administration	Samples After Taxanes	Range (In Days) After Taxane
0	191	63	22	106	4-966
1	21	2	6	13	8-287
2	4	1	0	3	14-405
3	**5**	**0**	**0**	**5**	**1-3**

The 5 samples with striking mitotic arrest mimicking HGD in nonneoplastic mucosa were from gastric antrum, cardia, oxyntic mucosa, appendix, and esophagus of 5 separate patients. All 5 had GIT samples obtained 1-3 days after taxane administration. On follow-up, in 3/5 patients with samples 1 day post treatment, 1 had acute appendicitis (died 180 days post bx), 1 died a day later of metastases, and 1 was asymptomatic (alive with metastatic disease at 126 days post bx). The remaining 2 died of metastases at 90 and 210 days post bx with no signs of drug toxicity at any time.

Conclusions: In contrast to colchicine-associated changes in nonneoplastic mucosa, the mitotic arrest mimicking HGD seen in GIT specimens after taxane administration is not specific for toxicity, but merely reflects taxane effect. It can be encountered in asymptomatic pts who have recently had medication. If these findings are seen histologically, they merit correlation with the clinical impression, and should not be interpreted as toxicity in isolation.

500 The Hippo Pathway in Human Esophageal Dysplasia and Carcinoma: A Novel Oncogenic Pathway?

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Background: The Hippo pathway balances cell proliferation and death in *Drosophila* and consists of a kinase cascade that controls a transcriptional co-activator, Yorkie. The understanding of this pathway in vertebrates is unknown. Recently, amplification of the mammalian homolog of Yorkie, YAP, was detected at low frequency in human hepatocellular carcinomas. We wished to determine if this novel oncogenic pathway is active in human esophageal tumorgenesis.

Design: The expression of YAP was evaluated with immunohistochemistry of tissue microarrays from 76 patients. Non-dysplastic, dysplastic and malignant foci were represented on the tissue microarray. Cytoplasmic and nuclear staining was scored as 0=none, 1= <10%, 2 = 10-50% and 3 = >50% for the non-neoplastic, dysplastic and malignant epithelium. Multiple scores were averaged for each patient. Fishers exact test was used as a test for significance.

Results: We found cytoplasmic YAP staining (>1.5) in the basal epithelium of non-neoplastic tissue. There was an increase in YAP staining in Barrett esophagus and low grade dysplastic epithelium, however, this increase was not statistically significant (Table 1). There was a significant increase in YAP cytoplasmic staining of high grade dysplastic epithelium and carcinoma. Each of the patients with YAP staining (>1.5) in the non-neoplastic epithelium also had a carcinoma. Nuclear staining showed a similar trend.

YAP Staining

Cell type	Cytoplasmic Staining	Nuclear Staining
Non-neoplastic squamous epithelium	11/54 20%	11/54 20%
Barrett Esophagus	3/21 14% p<0.40	4/21 19% p<0.5
Low Grade Dysplasia	6/17 35% p<0.17	1/19 6% p<0.1
High Grade Dysplasia	15/31 48% p<0.01*	14/31 45% p<0.01*
Carcinoma	25/65 38% p<0.05*	21/65 32% p<0.05*
Metastatic	8/29 28% p<0.31	8/29 28% p<0.31

p values relative to non-neoplastic mucosal epithelium, * = significant

Conclusions: The Hippo pathway is an important growth control mechanism in *Drosophila*, however, it has not been studied in vertebrates. YAP, the mammalian homolog of a transcriptional co-activator is part of this pathway. YAP expression in the cytoplasm and nucleus is significantly increased in high grade dysplasia and carcinoma. YAP expression in the cytoplasm of non-neoplastic tissues correlated with the presence of carcinoma. Our studies suggest a role for this recently uncovered oncogenic pathway in esophageal tumorgenesis.

501 Diagnostic Morphological Features of PDGFRA-Mutated Gastrointestinal Stromal Tumors

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Background: In 2003 activating mutations of PDGFRA (platelet-derived growth factor receptor alpha) were detected in a significant portion of KIT-negative GISTs. Since then, PDGFRA-mutated GISTs have been shown to posses several possible morphological signs distinguishing them from c-kit-mutated tumors at the light microscopic level. The purpose of this study was to evaluate the relation of all these promising markers to mutational status of c-kit and *PDGFRA*.

Design: Sixty specimens of GISTs were evaluated for the presence of multinucleated giant cells, rhabdoid cells, tumor infiltrating lymphocytes and mast cells, and edematous to myxoid stroma. DNA for molecular genetic investigation was extracted from formalin-fixed, paraffin-embedded tissues. Mutational analysis of exons 9, 11, 13 and 17 of the c-kit gene and exons 12, 14 and 18 of the *PDGFRA* gene was performed using PCR and direct sequencing. Then, relations of these mutations to the above mentioned histological features were evaluated.

Results: The patients included 27 men and 33 women with a mean age of 63,8 years (range 12 to 92). In total, 31 tumors were found to be c-kit mutated, 22 *PDGFRA* mutated, and 7 tumors to be wild type. Histological features evaluated as possible signs discriminating between *KIT* and *PDGFRA* mutated GISTs are shown in Table 1. The occurrence of studied histological features in *PDGFRA* mutated and *PDGFRA* wild type groups of GISTs is shown in Table 2.

Conclusions: Epithelioid or mixed epithelioid/spindle cell pattern and mast cell infiltration were found as the most reliable signs of *PDGFRA* mutation. Rhabdoid cells and multinucleated giant cells seemed to be less specific but still helpful markers in our study. Finally, tumor infiltrating lymphocytes and myxoid stroma do not seem to be valuable histological signs.

Table 1

Histological feature (100%)	KIT	PDGFRA	wt
Epithelioid component	13,3%	73,3%	13,3%
Myxoid stroma	37,5%	46,9%	15,6%
Mast cells	0	95%	5%
Multinucleated cells	25%	65%	10%
TILs	38,5%	48,7%	12,8%
Rhabdoid cells	25%	62,5%	12,5%

TILs: tumor infiltrating lymphocytes

Table 2

Histological feature	Non-PDGFRA (100%)	PDGFRA (100%)
Epithelioid component	20,8%	100%
Myxoid stroma	46,8%	68,2%
Mast cells	2,6%	86,5%
Multinucleated cells	18,2%	59,5%
TILs	52%	86,5%
Rhabdoid cells	23,4%	68,2%

TILs: tumor infiltrating lymphocytes

502 Instability of EGFR Gene Status during Progression of Colorectal Cancer

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Background: Advanced colorectal cancer (CRC) may be treated with epidermal growth factor receptor (EGFR) inhibitors. Patients can be selected for this treatment based on the EGFR status of their primary tumor as determined by immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH). The underlying assumption is that the EGFR status of the primary tumor is conserved in its local recurrence or metastases. The validity of this assumption has not been questioned up till now. It is also unclear which method (IHC or FISH) is the best predictor for response to treatment.

Design: We determined EGFR gene status by IHC and FISH in paired tumor biopsies of 31 CRC patients with either: 1) a primary and at least one distant metastasis or local relapse; or 2) two metachronous distant metastases. IHC was deemed positive if > 10% of tumor cells showed membranous staining above the background level. FISH was considered positive when there were ≥ 4 copies of the EGFR gene in ≥ 40% of the tumor cells, or when gene amplification was detected in ≥ 10% of the tumor cells. EGFR gene status was compared between primaries and their relapses or metastases. In addition, the results obtained by IHC and FISH were compared.

Results: 5/17 (29%) primary tumors and 2/13 (15%) metastases or local recurrences were positive for EGFR by IHC. These proportions increased to 42% and 22%, respectively, when examined by FISH. The first of 2 metachronous distant metastases was positive by FISH in 1/7 cases (14%). The next metastasis was positive in 4/3 (57%) of cases. EGFR status determinations by IHC and FISH were concordant in 17 patients and discordant in 9.

Conclusions: Our results suggest that EGFR gene status as determined by IHC or FISH is not stable during CRC progression. This is important in the context of recently published studies indicating a putative role for EGFR gene copy numbers in the primary tumor as a predictor of response to anti-EGFR antibodies (Moroni et al, Lancet Oncol 2005; Lievre et al, Cancer Res 2006). Our assessment of EGFR status by IHC and FISH highlights major discrepancies in nearly 35% of the cases. Further studies are needed to determine whether this is due to the several pre-analytical variables that flaw IHC analysis or to a differential transcriptional activity of the EGFR gene.

503 Tissue Transglutaminase II, a Key Enzyme Involved in Ulcer Healing in Ulcerative Colitis

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Background: Tissue Transglutaminase II (TGase) mediates transamidation of post-translational modification of proteins and is associated with cell migration, extracellular matrix assembly, cell apoptosis and differentiation. However, there is little study on (1) TGase expression in ulcerative colitis and (2) if it is involved in epithelial migration and ulcer healing. We analyzed the expression of TGase immunohistochemically in ulcerative colitis in both human and animal model and tested the effect of prostaglandin E2 (PGE2) and COX2 inhibitor, celecoxib, on TGase expression in animal model and intestinal cell line.

Design: 20 colectomy specimen with active chronic ulcerative colitis were analyzed from 2004 to 2006. Majority (18 cases) had been diagnosed as chronic ulcerative colitis with moderate to marked activity . Six cases had sections from apparently normal colonic mucosa. Paraffin sections were labeled with TGase antibody (Biocompare, New York) using heat-treated antigen retrieval and appropriate positive and negative controls. Dextran sulfate sodium (DSS) induced ulcerative colitis (UC) in mice and intestinal epithelial cell line IE6 were used to test the effect of PGE2 and celecoxib on TGase.

Results: Variable degree of TGase expression in the cytoplasm of regenerative epithelium, adjacent to ulcer in ulcerative colitis was identified in 17 of these 20 cases, as compared to no TGase expression in apparently normal colonic mucosa in 6 cases. In the ulcer areas, endothelial cells and a few subepithelial stromal cells displayed TGase expression. In DSS-induced UC in mice, TGase expression in regenerative epithelia in the ulcer area was also observed. With celecoxib treatment, larger ulcer formation and delayed ulcer healing were identified in mouse model. Significant decreased TGase expression in regenerative epithelia in the ulcer areas was found. Intestinal epithelial cell line IE6 with PGE2 treatment showed dose and time dependent induction of TGase expression using a Western blot approach.

Conclusions: We have demonstrated overexpression of Tgase in regenerative epithelia and endothelial cells adjacent to ulcer, suggesting its role in ulcer healing and angiogenesis. Animal model study further confirms these observations in human UC and identifies that PGE2 is a factor involved in the regulation of Tgase expression. This study implies that Tgase is a potential target for inflammation and ulcer healing. (Supported by NIH R01 CA104741).

504 Isolated Cecal Focal Active Colitis (FAC) in the Asymptomatic Adult: A Clinical, Endoscopic and Pathological Study of 24 Cases

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Background: FAC, a histologic term, describes the isolated finding of focal crypt injury. An infectious etiology accounts for the majority of cases. In a minority of cases, FAC may be a harbinger of Crohn's colitis. We have frequently noted asymptomatic individuals with FAC limited to the cecum. We present herein the clinical, endoscopic, histologic, as well as the outcome of such a cohort of cases.

Design: We identified asymptomatic individuals from our files between 1993 and 2006, with histologically documented FAC confined to the cecum. FAC was defined as the presence of at least a single crypt infiltrated by neutrophils. The clinical, drug, and endoscopy records were reviewed. Endoscopic and histologic features associated with bowel preparation artifacts were sought. Exclusionary histologic diagnoses included ischemic colitis, inflammatory bowel disease, granulomatous and pseudomembranous colitis. Cases in which a hyperplastic or adenomatous polyp was identified within the cecum were excluded. The inflammation was graded as follows: grade 1- cryptitis involving single fragments and less than five crypts; grade 2- cryptitis > grade 1; grade 3- grade 2 and mild basal plasmacytosis and/or misshapen or forked crypts.

Results: This study group was composed of 23 patients (9 M, 15 F) ranging in age from 36 to 79 (mean 56). The indications for colonoscopy were: screening (n=14), occult blood in stool (n=7) and iron deficiency anemia (n=2). On endoscopy the findings were restricted to the cecum and included erythematous patches (n=13), congestion (n=2), polypoidal mucosa (n=5) and ulceration (n=2). Periappendiceal involvement was noted in four cases. FAC grades were as follows: grade 1 in 10 cases; grade 2 in 9 cases; and grade 3 in 4 cases. Notably neither macroscopic aphthoid ulcers nor basal apoptosis, mucin depletion and epithelial sloughing suggestive of bowel preparation artifacts were seen. 6 of the 9 patients on whom a comprehensive drug history was available reported NSAID use. None developed inflammatory bowel disease (median follow-up 33 months, range 1 to 141).

Conclusions: Cecal endoscopic abnormalities, predominantly erythematous patches are occasionally identified in asymptomatic patients screened for colon cancer. Cecal FAC does not appear to represent a bowel preparation artifact. Etiological factors for further study include NSAID and secondary involvement by an appendiceal process. Importantly these lesions appear neither to be clinically significant nor harbingers of inflammatory bowel disease.

505 Duodenal Intraepithelial Lymphocytosis Is Not Associated with *Helicobacter Pylori* Gastritis in the Pediatric Population

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Background: Duodenal intraepithelial lymphocytosis with preserved villous architecture has been associated with *H. pylori* gastritis in the adult population and is recognized as a potential histologic mimic of celiac disease. We have investigated this in a pediatric population with biopsy proven *H. pylori* gastritis.

Design: Patients with concurrent antral and duodenal biopsies were found by searching the pathology records at Children's Hospital Boston, Boston, MA between June, 2002 and June 2006. The study group included 59 patients with *H. pylori* gastritis (59 cases); control populations included celiac disease (CD; 7 cases), upper gastrointestinal tract Crohn's disease (CR; 12 cases), chronic inactive gastritis with negative *H.pylori* immunohistochemistry (CG; 14 cases), and 7 cases with normal histologic findings. The antral biopsy, duodenal biopsy, and *H. pylori* immunohistochemical stain (if available) on each case were studied in a blinded fashion. Duodenal biopsies were evaluated for number of intraepithelial lymphocytes (IELs) per 100 enterocytes, distribution of IELs along the villi, villous architectural changes, foveolar metaplasia, and increased lamina propria chronic inflammation. The antral biopsies were assessed using the revised Sydney criteria; additionally, the number of surface IELs were counted in the antral biopsies. Statistical analysis was performed using the Mann-Whitney test.

Results: The mean patient age for all biopsies was 12 years, and the male to female ratio was 1.2:1. The numbers of duodenal IELs were not significantly different when the *H. pylori* group (mean 21.9 +/- 14.0) was compared to the normal (18.0 +/- 5.0), CR (23.3 +/- 6.9), and CG (20.6 +/- 10.0) groups. However, the numbers of duodenal IELs were significantly increased in the CD (47.7 +/- 21.0) group as compared to the normal, and *H. pylori* groups. The remaining variables in both the duodenal and antral biopsies did not correlate with an increase in duodenal IELs. However, villous atrophy correlated with increased IELs in the CD group.

Conclusions: Our study found no significant increase in the number of duodenal IELs in pediatric patients with *H. pylori* gastritis. This finding is in contrast to the recently reported association between increased duodenal IELs in adults infected with *H. pylori*.

506 The Significance of Diverticular Disease of the Vermiform Appendix in Surgical Pathology

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Background: Acquired diverticula of the vermiform appendix are rare and arise from different pathogenetic mechanisms. One of the etiologies includes proximally located, often unsuspected small neoplasms. Although the association of appendiceal diverticulosis and neoplasia is known it remains underemphasized in the teaching and practice of surgical pathology.

Design: To investigate the frequency of appendiceal neoplasms with acquired diverticulosis we conducted a retrospective analysis of all appendectomy specimens received in our institution over a 55 month period (Jan 2002 – July 2006).

Results: A total of 1361 appendectomy specimens were identified. Pathologic diagnoses for these cases included acute suppurative appendicitis in 1102 (81%) cases and primary appendiceal neoplasm in 29 (2.1%) cases. The remaining 230 (16.9%) cases were diagnosed with various pathologies (eg: fecalith, endometriosis and parasitic infestations). Diverticulosis was diagnosed in 23 (1.7%) of all cases. Eleven (44%) appendectomy specimens with diverticulosis also harbored an appendiceal neoplasm. Neoplastic processes included: 5 well differentiated neuroendocrine tumors (carcinoids), 3 mucinous adenomas, one tubular adenoma and two adenocarcinomas. In one case, routine representative sections sampled only a small focus of carcinoma which originally went undiagnosed.

Conclusions: We stress the need for meticulous gross assessment with histological examination of the entire appendectomy specimen in cases of appendiceal diverticulosis. Thorough examination is required to rule out an underlying neoplasm as a cause of diverticulosis. As acquired diverticula represent a rare finding, examination of the entire appendix in this setting does not create a significant impact on the workload within the pathology laboratory.

507 Primary Colorectal Small Cell Carcinoma: A Clinico-Pathological and Immunohistochemical Study of 9 Cases

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Background: Colorectal small cell carcinoma (SCC) is a rare tumor with an aggressive course and a fatal outcome. The aim of this study is to summarize our experience with this rare tumor and to highlight its immunohistochemical profile.

Design: Surgical pathology database and medical records of Sunnybrook Health Sciences Centre were searched in the period of July 1999 to June 2005. Nine cases of colorectal SCC were identified and a panel of immunostains was performed.

Results: Cases ranged from 42 to 88 years old with a mean age of 60.1 years, and showed female: male ratio of 2:1. One case had a history of familial colorectal cancer, 2 had IBD and 3 were associated with colonic adenomas. Eight patients presented with symptoms related to a colonic mass and 6 had metastases at the time of diagnosis. Four cases were treated by surgical resection with adjuvant chemotherapy, one was diagnosed in post-mortem examination and 4 had palliative chemotherapy following a biopsy diagnosis. Follow up was available for the average of 3 years, during which 5 patients died and the remaining four were alive with disease. All cases were positive for LMWK, CK 19 and pankeratin but were negative for TTF-1. CDX2 was positive in only one case while D14 CEA was positive in another. Results of the other immunostains are shown in the table below.

Conclusions: Colorectal SCC is an aggressive tumor with a predilection for females, old age, and rectum. The tumor is consistently positive for LMWK, CK 19 and pankeratin while CK 20 expression is site-dependent, with negative staining in all rectal tumors. The tumor's TTF-1 negative staining is valuable in differentiating it from SCC of a pulmonary origin. CDX2, D14 CEA, CD56, and NSE help differentiate it from poorly differentiated colorectal adenocarcinoma. The expression of EGFR in a subset of patients could have a role in the future planning of targeted biologic therapy.

Immunostains of 9 cases of primary colorectal SCC

Case: Age/Sex/Site	CK7	CK20	Synaptophysin	Chromogranin	CD56	NSE	EGFR
65/M/ileocecal	-	+	+	+	+	+	+
69/M/Ascending colon	-	+	+	-	+	+	-
88/F/Ascending colon	+	-	-	-	+	-	+
60/M/Splenic flexure	-	+	+	+	+	+	-
42/F/Rectum	-	-	+	-	+	+	-
43/F/Rectum	+	-	-	-	+	-	+
49/F/Rectum	+	-	-	+	-	+	+
59/F/Rectum	-	-	+	+	+	-	+
66/F/Rectum	-	-	+	+	+	+	-

508 The Rectal Tonsil: Analysis of Salient Histologic Features To Recognize This Important Entity

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Background: The rectal tonsil is an entity that has been recognized for a number of years but can still cause diagnostic difficulty when encountered in practice. Awareness of this entity can prevent an unwarranted diagnosis of an atypical lymphoid proliferation or lymphoma. Retrospective analysis of cases of the rectal tonsil was performed in order to determine characteristic features which can aid in the recognition of the rectal tonsil.

Design: Nine cases of rectal tonsil 1996-2005 were collected. The cases were all examined using H & E stained sections, and immunohistochemistry was used in problematic cases. Histologic features assessed included: the expansile nature of the lymphoid infiltrate, the presence of lymphoepithelial lesions, and the integrity of the crypts. When available, the presence of follicles was determined using anti-follicular dendritic cell (FDC) immunohistochemistry (CD21, CD23). Follow-up data was collected on all cases.

Results: Endoscopic data, available in 8 cases, referred to a polypoid lesion in 6 cases and a mass or nodular lesion in 2 cases. All cases were composed of a submucosal and/or lamina propria lymphoid infiltrate. Lymphoid follicles could be identified in all cases, although some were difficult to appreciate without FDC markers because of biopsy crush artifact. The lymphoid infiltrate was dense in 8/9 (89%) of cases and focal in 1/9 (11%) of cases. In cases where germinal centers were easily visible, tingible body macrophages were noted. Prominent stromal sclerosis was present in 2 cases. Five cases showed overlying cryptitis and mild architectural distortion, but no cases showed crypt obliteration or crypt abscesses. Intraepithelial lymphocytes were present in 8 cases, and 5 cases showed lymphoepithelial lesions. No cases showed recurrence after an average of 4.8 years (range: 1-10 years). One case was initially worrisome for lymphoma but had noticeable follicles with CD21 staining, and was benign on re-biopsy.

Conclusions: The rectal tonsil is an important entity to recognize. Noticable features include a dense nodular lymphoid infiltrate of the submucosa and/or lamina propria and the presence of follicles which may be distorted by biopsy crush artifact. However, the follicles are not disrupted or colonized by extrafollicular lymphoid cells. Utilizing an approach which evaluate these features can help distinguish the rectal tonsil from clonal lymphoid proliferations but rebiopsy and/or gene rearrangement studies should be performed if in doubt.

509 Sequential Analyses of the Growth of Activated Stromal Foci with Increased Expression of CD34, CD68, α-Smooth Muscle Actin, and COX-2 in the Rat Colon Carcinogenesis: Putative Markers of Field Cancerization and Relationship with Increased Crypt Fission Rates

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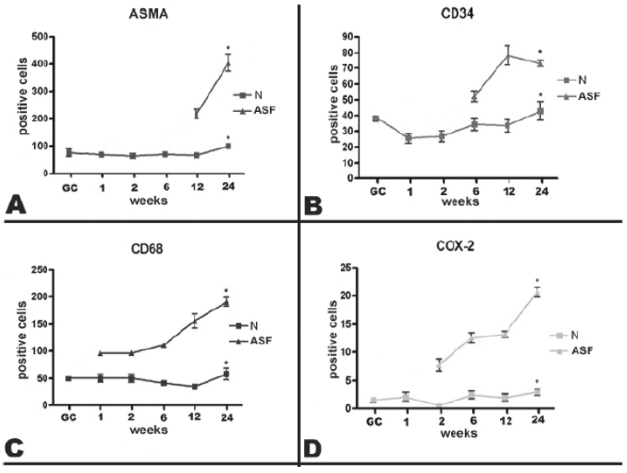
Background: It is now well accepted that the coordinated activity of epithelial cells with their stroma is fundamental in controlling growth and differentiation in normal and pathological situations, including cancer. A number of reports stress the importance of the stromal compartment in malignant tumors and strongly indicate that continuous interactions between the carcinoma and stromal cells (resulting in their reciprocal regulation and modulation) are prerequisites for carcinoma development and progression. Comparatively, less information is available about the features and role of the stroma for the carcinogenic process.

Design: Fourty-eight male Wistar rats received a single i.p. injection of 125 mg Dimethyl-hydrazine per body weight and subdivided into five groups according to the sacrifice time (1, 2, 6, 12 and 24 weeks, after receiving the carcinogen injection). The control group received an injection of saline and was euthanized after 12 weeks. Immediately after the sacrifice, colon samples were removed and submitted to a standard avidin-biotin immunohistochemistry method with the antibodies CD34, α-smooth muscle actin (SMA), CD68 and COX-2.

Results: In animals treated with the carcinogen Dimethyl-hydrazine we identified the appearing of mucosal "activated stromal foci" (ASF) that differ from the stroma and sporadic inflammatory foci found in the normal mucosa of the control animals because of the presence of increased immune-expression of CD34, CD68, α -actin, COX-2 and microvessel density. Furthermore, the ASF surrounded a increased number of colonic crypts in fission when compared to areas of normal stroma. This last finding suggests that stromal activation and epithelial changes may be correlated during the colonic carcinogenesis.

Conclusions: Taken together with literature data, our findings suggest that in the colon, the epithelial field cancerization may be accompanied by stromal changes and this may point to the finding of new markers of neoplastic transformation and field cancerization of the colon.



510 Application of Fluorescence In Situ Hybridisation to the Pre-Operative Diagnosis of Pancreatic Lesions: A Preliminary Study

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Background: Pancreatic adenocarcinoma is the fourth most frequent cause of cancer death in Western countries. Due to the non-specific nature of the symptoms 80% of these tumors are not resectable at diagnosis. Recent advances in interventional radiology now permit preoperative cytological diagnosis of pancreatic lesions, but poor sensitivity (29-56%) has limited the large scale use of these techniques. Numerous genetic abnormalities have been identified in pancreatic adenocarcinoma, amongst others, deletions in p53, SMAD4 and p16 as well as amplification of c-myc are frequent. The overall aim of this study is to use fluorescence *in situ* hybridization (FISH) to differentiate between neoplastic and non-neoplastic pancreatic lesions before applying this technique to cytological specimens.

Design: In this initial phase formol fixed paraffin embedded blocks from 10 pancreatic adenocarcinomas, 9 cases of chronic pancreatitis and 5 morphologically normal pancreatic tissue excised with cystic lesion (mucinous or serous cystadenomas) were investigated. FISH analysis was undertaken using commercially available probes targeting p53 (17p12.1), SMAD4 (18q2), p16 (9p21) and c-myc (8q24) (Vysis®). The percentage of cells bearing chromosomal deletions of the regions of p53, SMAD4 and p16 and amplifications of the c-myc regions were evaluated.

Results: See table 1. All cancers with genetic modification (8/10) bore at least two abnormalities a molecular phenotype that was never present in non-neoplastic lesions.

Conclusions: The presence of at least two genetic lesions detectable by FISH analysis of p53, p16, SMAD4 and c-myc allows adenocarcinomas to be distinguished from non-neoplastic lesions with a sensitivity of 80% and a specificity of 100%. This study will be expanded to a larger patient group and applied to cytological diagnosis using a microcroscopy technique that allows identification of atypical cells and subsequent re-evaluation of the same cells following FISH. We expect this approach to provide a significant increase in the sensitivity and specificity of cytological diagnosis.

results : percentage of samples showing genetic abnormalities

	Adenocarcinoma	non-neoplastic lesions
p53	45,5	15
p16	66,6	9
SMAD4	80	0
c-myc	40	0

511 Decreased CK20 Expression in MGMT-Deficient Colorectal Carcinomas: Evidence of a Distinct Phenotypic Association

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Background: O^6-methylguanine-DNA-methyltransferase (MGMT) is a DNA repair enzyme that removes DNA damage-associated alkyl groups, and that is commonly silenced in a variety of cancers, including colorectal cancers (CRC). We hypothesized that the increased mutagenesis in tumor cells with MGMT loss may result in a specific mutator phenotype that could drive a distinctive pathway of colorectal tumorigenesis. We therefore examined the expression patterns of characteristic immunophenotypic markers CK7, CK20 and CDX-2 in a series of MGMT-intact or -deficient CRC.

Design: We have previously tested a consecutive series of CRC for expression of MLH1 and MGMT, and classified them as MLH1-deficient/MGMT-intact (MLH1-D), MLH1-intact/MGMT-deficient (MGMT-D) or MLH1-intact/MGMT-intact (Intact); tumors that were both MLH1-D and MGMT-D were excluded from the present study. Routinely processed tissue sections from 20 of each of these 3 categories of tumors were stained with CK7, CK20 and CDX-2. The extent of reactivity was graded according to the percentage of positive tumor cells: 0, no staining; 1+, <5%; 2+, 5-25%; 3+, 26-50%; 4+, 51-75%; 5+, 76-100%.

Results: The mean score for CK20 staining was 3.9 (+/-1.6) in Intact CRC, compared to 2.0 (+/-1.4) in MGMT-D tumors (p=0.0001) and 1.7 (+/-1.6) MLH1-D tumors (p=0.0004). The overall percentage of CK20 positive tumors was as follows: 90% in Intact tumors, 95% in MGMT-D tumors, and 75% in MLH1-D tumors. CK7 staining did not vary significant between the tumor groups (mean scores of 0.3 +/-0.6, 0.3 +/-0.7, and 0.6 +/-0.9, in Intact, MGMT-D and MLH1-D tumors, respectively), though there was a trend toward increased CK7 expression in MLH1-D (p=0.10). The overall percentage of CK7 positive tumors was 20% in Intact tumors, 16% in MGMT-D tumors, and 35% in MLH1-D tumors (p=0.16 for MLH1-D vs MGMT-D). CDX-2 staining was detected in 100% of all CRC types; the mean scores were 4.6 +/-1.1, 4.8 +/-0.9, and 4.5 +/-1.1 in Intact, MGMT-D and MLH1-D tumors, respectively.
Conclusions: Our findings confirm previous observations that MLH1-D CRC have significantly reduced CK20 expression compared to MLH1-intact CRC. In addition, we have found that MGMT-D CRC are also characterized by a significant reduction in CK20 expression compared to intact CRC. These findings support a major alteration in differentiation pathways in MGMT-D CRC, and support the hypothesis that MGMT-D CRC may evolve along a distinctive pathogenetic pathway compared to tumors with intact MGMT expression.

512 Pseudomelanosis Duodeni: Association with Multiple Clinical Conditions and Unpredictable Iron Stainability

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Background: Pseudomelanosis duodeni is a rare enigmatic condition characterized by endoscopic detection of dark spots in the duodenal mucosa. Conventionally regarded as a direct effect of therapeutic oral iron intake and local deposition of iron in the duodenal mucosa, occasional absence of oral iron intake and the frequent lack of partial or total iron stainability appear perplexing. Some electron-probe x-ray studies have shown variable admixture of non-stainable ferrous sulfide and stainable hydrated iron oxide. This study is undertaken to highlight the multiple clinical conditions and the unpredictable iron stainabiltiy asssociated with pseudomelanosis duodeni.
Design: Between 1996 and 2006, 14 adult patients (9F, 5M, mean age 62) with endoscopic and histological diagnosis of pseudomelanosis duodeni were identified in our files. Their clinical history including oral iron intake was reviewed. The duodenal biopsies were stained for iron (Perls), copper (Rhodanine) and calcium (Alizarin Red). 15 control patients with history of oral iron intake and duodenal biopsies were also reviewed.
Results: 43% of patients had no history of oral iron intake. Hypertension (86%), end stage renal disease (57%), diabetes mellitus (57%) and oral iron intake (57%) were conditions associated with duodenal pseudomelanosis. Perls iron stain was entirely positive in 37%, only partially positive in 43% cases and totally negative in 20% cases. Copper and calcium stains were negative in all cases. No iron deposition was seen in 15 control patients with iron intake.
Conclusions: Pseudomelanosis duodeni is a complex phenomenon associated with multiple clinical conditions such as hypertension, renal disease and diabetes and not incontrovertibly oral iron intake. Most patients with sole history of oral iron intake do not display this condition. The iron stainability of the pigment is variable and unpredictable. Further studies are warranted in assessing selective duodenal mucosal vulnerability for deposition of this complex pigment in disparate but distinct clinical conditions.

513 Morphologic Features of High-Grade Serrated Dysplasia in Sessile Serrated Adenomas without Coexistent Invasive Adenocarcinoma

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Background: The cytologic features of high grade serrated dysplasia (HG-SD) in SSAs with coexistent adenocarcinoma was recently described. We studied the prevalence and the morphologic features of SSAs with HG-SD unassociated with adenocarcinoma.
Design: 528 morphologically clear-cut SSAs (>50% dysmaturational crypts) from the daily case load of one author with an interest in GI pathology during Jan, 2003 through March 2005 were reviewed. Polyp size, location, presence, extent, and features of HG-SD was recorded. SSAs ranged in size from 4mm to <2cm. All were removed by endoscopy including the large polyps. High grade dysplasia was defined as epithelial cells with frankly malignant cytologic features.
Results: 24 of the 528 SSAs had HG-SD (4.55%), including 4/148 (2.7%) 5mm polyps, 6/171 (3.51%) 6mm polyps, 4/70 (5.71%) 7mm polyps, 4/31 (12.90%) 8mm polyps, and 6/27 (22.22%) 1-2 cm polyps. The mean number of crypts with HG-SD was 7.3(range 5 – 28).The number of HG-SD crypts per polyp was similar in polyps of all sizes and did not increase in proportion to polyp dimension. Crypts with HG-SD were usually closely clustered and had moderately dilated or flask-shaped crypt bases. HG-SD cells were predominantly located in the basilar and mid crypt regions of dysmaturational crypts with the most dysplastic cells located in the basilar crypt region. There was slight to marked cell "maturation" as the HG-SD cells extended upwards towards the surface HG-SD cytologically consisted of monomorphic, cuboidal to slightly elongate cells with round or oval nuclei, pale (open) chromatin, and prominent nucleoli.
Conclusions: High grade serrated dysplasia is uncommon, occurring in less than 5% of SSAs. SSAs of all sizes can have HG-SD. However, large SSAs had a much higher prevalence of HG-SD than small SSAs (22% vs 3%). HG-SD develops in the basilar crypt regions, often in a cluster of highly dysmaturational crypts with dilated crypt bases. Similar to dysplasias from other regions of the GI tract, such as the lower esophagus, dysplastic cells tended to show maturational changes as they extended along crypts towards the surface.

514 "Advanced" Adenomas: Pathologists Don't Agree

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Background: Several major cancer and gastroenterologic societies have published guidelines for surveillance intervals following colorectal adenoma removal. The interval is shortened from 5 to 3 years when there are more than 3 adenomas or when an "advanced" adenoma (size >/= 1cm, presence of villous architecture and/or high-grade dysplasia) is identified. Histologic criteria for these morphologic features are not defined in the guidelines. If the guidelines are to be valid, then all pathologists who routinely evaluate adenomas should uniformly characterize villous architecture and high-grade dysplasia. To our knowledge, there is no study examining the reproducibility of these determinations.
Design: Twenty-one adenomas of varied size and architectural complexity were submitted for evaluation to 30 pathologists in 6 practices. They were asked to determine if each adenoma had a villous component and/or high grade dysplasia. No definitions of these features were given, in keeping with the guidelines above. Each pathologist was asked to identify her/himself as a gastrointestinal or general pathologist. We also requested an assessment of the percent of adenoma surface with villous architecture. The data were evaluated for percent agreement among the participants.
Results: Nineteen gastrointestinal pathologists and 11 general pathologists participated in the study. Among all pathologists, >/= 90% agreement was achieved for only 10 of 21 adenomas (48%) with respect to villous architecture, and for 13 of 21 adenomas (62%) with respect to high-grade dysplasia. At >/= 80%, the agreement was slightly increased to 67% and 71%, respectively. Of the 21 adenomas examined, the number identified as having villous architecture ranged from a low of 5 by one reviewer to a high of 17 by another (Kappa = 0.4924, p = 0.0000), and for high-grade dysplasia from 0 to 14 (Kappa = 0.4363, p = 0.0000). Estimates of percent surface with villous architecture varied widely, ranging from 2 to 100%, even among those responses for adenomas carrying >/= 90% agreement for presence of villous features. Specialized gastrointestinal pathologists agreed less on adenomas with villous architecture and/or high-grade dysplasia than did general pathologists.
Conclusions: Identification of villous architecture and high-grade dysplasia in adenomas has poor reproducibility among pathologists, irrespective of subspecialty training. These results raise serious questions about endorsing such follow-up criteria in patients with adenomas.

515 Pathologic and Molecular Features of Colorectal Carcinoma (CRC) in Young Patients

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Background: The incidence of CRC among young patients has risen by nearly 20% in recent decades, but potential reasons for this increase are not known. The aim of this study was to identify any clinical, pathologic or molecular differences between younger and older CRC patients, in order to shed light on this phenomenon.
Design: Pathologically staged CRC cases from 22 [male/female (M/F): 10/12] patients <40 years of age and 27 (M/F: 14/13) controls >50 years of age were evaluated for the presence of 1) CRC risk factors [family cancer history in a 1° relative, tobacco use, polyposis syndromes, ulcerative colitis (UC)], and 2) pathologic predictive features of outcome [stage, grade, lymphatic (LI) or venous (VI) invasion, Crohn's-like response, tumor regression after neoadjuvant therapy]. The cases were immunostained for MLH1, MSH2, MSH6, p53, β-catenin, EGFR, p16, and AMACR, an enzyme involved in branched-chain fatty acid metabolism. MSI analysis was performed by subjecting tumor DNA extracts to multiplex PCR amplification of 5 mono- and 2 pentanucleotide markers. MSI of ≥2 markers was considered MSI-H.
Results: Of patients <40 years of age, 1 each had HNPCC, FAP, and UC, and were excluded from further analysis. Thus, 19 patients comprised the study group (SG). Of these, 2 (11%) had a family cancer history, compared to 11 (41%) controls (p=0.05). All SG tumors occurred in the distal colon and 68% were in the rectum, compared to 56% (p=0.001) and 22% (p=0.003) of controls. Most SG tumors were stage III or IV (95% vs. 59%, p=0.05), and more frequently showed VI (59% vs. 19%, p=0.009) or LI (88% vs. 63%, p=0.07) than controls. AMACR expression was significantly more common in the SG (94% vs. 41%, p<0.0005), which also showed a trend toward β-catenin (65% vs. 37%, p=0.07) and p53 expression (77% vs. 52%, p=0.09) compared to controls. EGFR (35% vs. 48%) and p16 (82% vs. 96%) staining was similar in both groups. None of the SG tumors and 11% of controls were MSI-H (p>0.05). No other differences between the groups were noted.
Conclusions: Compared to older patients, most young patients with CRC have high-stage, distal tumors without identifiable risk factors or MSI. Most CRC in young patients express AMACR, which *in vitro* studies have shown to be upregulated by exposure to branched-chain fatty acids present in red meat and dairy products. Thus, further studies evaluating potential dietary influences in young CRC patients may be warranted.

516 Increased Expression of γ-H2AX in the Proliferative Stem Cell Compartment of the Colon in Ulcerative Colitis: A Marker of DNA Damage

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Background: The increased risk of colon cancer in patients with ulcerative colitis (UC) may be due, in part, to DNA damage associated with increased proliferation and stem cell turnover that occurs with epithelial damage and repair. DNA double-strand breaks and shortened, dysfunctional telomeres that result from replicative stress induce a DNA damage response that includes phosphorylation of histone H2AX (γ-H2AX)

and recruitment of repair proteins to the site of DNA damage. We hypothesize that the replicative stress that occurs in UC will lead to activation of the DNA damage response and increased γ-H2AX expression in the basal epithelial cells, the location of colonic stem cells and transitional cells.
Design: Cases of UC (n=19) with varying levels of activity (inactive [2], minimal [3], mild [5], moderate [6], or severe [3]) and normal colonic controls (n=10) from uninvolved colon in cases of diverticulosis were selected from the Hospital of the University of PA Pathology archives and stained with a polyclonal antibody against γ-H2AX using standard immunohistochemical techniques. For each section, the total number of γ-H2AX-positive epithelial cells located within the base of the crypts, the total number of crypts that contained γ-H2AX-positive cells, and the total number of crypts evaluated were recorded. Results were expressed as # positive basal epithelial cells/crypt and % positive crypts. Statistical analysis was performed using the Mann-Whitney test and Spearman rank correlation.
Results: For cases of UC and normal colon, the median patient age was 39 and 60 years with a male to female ratio of 11:8 and 4:6, respectively. The median total number of crypts assessed in UC and normal colon were 266 and 672, respectively. Cases of UC had five-fold increased frequency of γ-H2AX expression compared to normal colon (median # positive basal epithelial cells/crypt of 0.027 vs 0.005, respectively, p=0.009; median % positive crypts of 1.9% vs 0.4%, respectively, p=0.0011). In UC cases, there was no significant association of γ-H2AX expression with disease activity and duration (median=11 years).
Conclusions: The increased frequency of γ-H2AX expression in the basal epithelial compartment in patients with UC compared to normal colon suggests that the replicative stress that occurs in this critical proliferative stem cell compartment results in DNA damage which may contribute to the increased risk of neoplasia in patients with UC.

517 sFRP-1 and Maspin Immunohistochemistry of Serrated Colorectal Polyps with Abnormal Proliferation

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Background: Recent work delineating a serrated polyp neoplasia pathway has led to a morphologic reappraisal of the traditional hyperplastic polyp (HP). Certain hyperplastic-like serrated polyps harbor genetic alterations that predispose them to malignant progression. The precursor HPs have been termed serrated polyps with abnormal proliferation (SPAP). Detailed morphological studies have identified subtle differences distinguishing the SPAP from conventional HPs, but ancillary markers are needed in diagnostically challenging cases. We investigated the staining characteristics of sFRP and Maspin to determine if they can provide assistance when trying to identify SPAPs.
Design: Using published morphologic criteria we reviewed H&E slides of 103 polyps from 80 patients and divided them into SPAP and conventional HPs. We then stained archival paraffin embedded tissue with sFRP-1 (Polyclonal, Eurogentec, Seraing, Belgium). and Maspin (Polyclonal, BD Biosciences, San Jose, CA). The staining pattern, particularly differing immunoreactivity between apical and basal colonic epithelial cells was evaluated. For sFRP-1, apical staining was scored as present or absent. For Maspin, relative staining intensity of apical and basal cells were scored as apical>basal, apical=basal, and apical<basal.
Results: Thirty-nine (36.4%) polyps met the criteria for SPAP. Polyps in the right (40.9%) and transverse (50.0%) colon had the largest percentage of SPAPs. The majority of SPAP (89.5%) and HPs (83.1%) showed apical sFRP-1 staining. Maspin staining showed moderate to strong cytoplasmic staining throughout the polyp colonic epithelium. However, differences in Maspin staining intensities were observed. A greater number of HPs (75.4%) showed apical>basal compared to SPAPs (59.1%). SPAPs (31.8%) were more likely to show uniform apical=basal staining than HPs (18.5%).
Conclusions: sFRP-1 does not appear to differentiate HPs from SPAPs. Maspin down regulation in the apical cells of SPAPs relative to basal cells may be implicated in the pathogenesis their pathogenesis. SFRP-1 or Maspin staining appears to be of no clinical utility to differentiate SPAPs from conventional HPs, but Maspin staining patterns corroborate a tumor suppressor role for Maspin in epithelial colorectal neoplasms.

518 Validation of Morphologic Predictors of Microsatellite Instability in Colorectal Cancer

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Background: The histopathologic recognition of microsatellite instability is clinically relevant for detecting defective mismatch repair arising through either germline mutation or promoter methylation. In a prior interim analysis of 528 population-based cases of colorectal cancer we quantified the strong relationship of tumor infiltrating lymphocytes, the lack of dirty necrosis, a Crohn's-like host response, right-sided location, well or poor differentiation, and any mucinous differentiation as predictors of high microsatellite instability (MSI-H). In the present analysis, we provide results for the completed study of pathologic predictors of microsatellite instability among 1,911 incident cases of colorectal cancer(CRC).
Design: As part of a population-based case control study of CRC in northern Israel, we reviewed the pathology and microsatellite status of 1911 consecutively diagnosed CRCs. Phenotypic analysis was performed by one pathologist (JKG) and included assessment of grade, mucinous histology (>50%, or focal), histologic heterogeneity, growth pattern, and host response. Microsatellite status was determined on microdissected portions of formalin-fixed paraffin-embedded tissue using a panel of 5 NCI consensus primers.
Results: 238/1911 cases (12.45%) demonstrated a high degree of microsatellite instability (MSI-H). Individually, tumor infiltrating lymphocytes, the lack of dirty necrosis, a Crohn's-like host response, right-sided location, well or poor differentiation, and any mucinous differentiation were predictive of MSI-H tumors, p<0.0001 for each pathologic predictor. In a multivariate logistic model, tumor infiltrating lymphocytes, well or poor differentiation, and any mucinous differentiation were the strongest independent predictors of MSI-H tumors.
Conclusions: As pathologists are increasingly relied upon to recognize MSI-H tumors based on histopathologic criteria for further workup with immunohistochemistry and/or molecular testing, prioritizing the features that are most strongly predictive of MSI is clinically valuable. As in our earlier study, we found a signficant number of MSI-H tumors that were well differentiated and only focally mucinous.

519 Expression of Melanoma Antigens in Epithelioid Gastrointestinal Stromal Tumors (GIST); a Potential Diagnostic Pitfall

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Background: The majority of gastric GIST express CD117/c-kit, as do a subset of metastatic melanomas, leading to a diagnostic dilemma in some cases. To further help differentiate GIST from melanoma, we investigated expression of melanoma markers in GIST using a well-characterized set of gastric lesions on tissue microarrays (TMAs).
Design: Paraffin-embedded tissue cores (169, including 135 GIST and 34 normal gastric wall smooth muscle from the same patients) from 38 patients were collected and used for the construction of TMAs. One to five 6-mm tissue cores were sampled from each patient specimen (average of three tissue cores/patient). The cases were arrayed on two TMA blocks. All cases had been previously stained with CD117/c-kit and CD34 antibodies. All were reactive with CD117/c-kit and 88.2% expressed CD34. Slides were stained with S100 protein, HMB-45, and Melan-A antibodies.
Results: S100 protein was focally expressed in 2/38 (5.3%) of GIST; these lesions lacked HMB-45 and Melan-A labeling. Over half of the cases (63.6%) demonstrated scattered S100+ interdigitating dendritic cells. No tumor labeled with HMB-45 but 4/38 (10.6%) cases labeled with Melan-A antibodies. The S100 reactive cases were spindle-cell neoplasms whereas the Melan-A reactive cases were epithelioid neoplasms. The Melan-A reactive cases were all CD34 positive. Labeling of neoplastic cells with S100, or Melan-A, or presence of S100+ interdigitating dendritic cells had no influence on survival outcome.
Conclusions: Melan-A staining can be encountered in a subset of epithelioid GIST, a finding that can suggest a differential diagnosis of melanoma. In this series, the Melan-A reactive cases lacked S100 protein and expressed CD34, both of which would be unlikely in melanoma. As such, a panel approach is best in differentiating epithelioid GIST from melanoma.

520 Non-Goblet (Cardia-Type) Epithelium in Barrett's Esophagus Is "Intestinalized": Implications with Regard to the Pathogenesis of This Disorder

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Background: Barrett's esophagus (BE) is defined by the presence of intestinal metaplasia (IM) containing goblet cells (GC), within endoscopically recognizable areas of the esophagus. However, some carcinomas in BE, or from the GEJ region, develop in mucosa devoid of GC. Furthermore, the biological properties, pathogenesis, and potential risk of malignancy of non-GC columnar epithelium in BE is essentially unknown. The aim of this study was to evaluate the biological properties of non-GC ("cardia-type") epithelium in the progression of IM in BE.
Design: Seventy routinely processed four-quadrant mucosal biopsies from well-defined levels of the esophagus, representing GEJ and non-GC epithelium from GC deficient, GC poor (1-2/4 quadrant biopsies with GC) and GC rich areas (3-4/4 quadrant biopsies with GC) in the esophagus, were obtained from 29 BE patients without dysplasia. All biopsies were stained immunohistochemically with antibodies to intestinal specific antigens and transcription factors (MUC1, MUC2, DAS-1, and CDX-2), and Ki 67, and the results were compared between different regions of the non-GC columnar epithelium in a blinded manner, without knowledge of the GC density in each level of the esophagus.
Results: MUC2, DAS-1 and CDX2 stained GEJ non-GC epithelium in 0%, 23%, and 8.0% of patients, respectively, and was increased significantly (p<0.01) in non-GC epithelium from esophageal mucosa deficient for GC (MUC-2: 25%, DAS-1: 75%, CDX-2: 25%) compared to esophageal mucosa with more abundant GC's (GC poor; MUC-2: 30%, DAS-1: 50%, CDX-2: 50% vs GC rich; MUC-2: 74%, DAS-1: 96, CDX-2: 100%). In addition, all markers (MUC1, MUC2, DAS-1, CDX-2) showed significantly increased staining in non-GC epithelium in GEJ mucosa with GC's compared to GEJ mucosa without GC's. Finally, significantly increased Ki 67 proliferative activity was noted in non-GC epithelium in biopsies with GC's, compared to those without.
Conclusions: These results provide strong evidence that non-GC (cardia-type) epithelium in BE is physiologically "intestinalized", and hyperproliferative. Thus, it may possess a potential risk of neoplastic progression, which needs to be tested in future studies that evaluates genetic and epigenetic abnormalties. GC metaplasia probably occurs as a secondary metaplastic event within a field of progressively "intestinalized" non-GC epithelium.

521 Immunoreactivity for CD25 in Gastrointestinal Mucosal Mast Cells Is Specific for Systemic Mastocytosis

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Background: Systemic mastocytosis (SM) is characterized by the accumulation of neoplastic mast cells in bone marrow and other organs. Gastrointestinal (GI) symptoms are common in both SM and cutaneous mastocytosis (urticaria pigmentosa, UP), usually caused by the release of histamine and other inflammatory mediators. Occasionally,

neoplastic mast cells may also directly infiltrate the GI tract. Past studies have suggested that enumeration of mast cells in GI biopsies may help establish the diagnosis of SM. However, mast cells are increased in various inflammatory diseases, and mast cell density has not been systematically evaluated in other GI disorders. Recently, expression of CD25 by mast cells in bone marrow has been shown to be specific for SM. The purpose of this study was 1) to quantitate and compare mast cells in mucosal biopsies from patients with SM involving the GI tract, UP with GI symptoms, and a control group of diverse inflammatory disorders, and 2) to determine whether immunostaining for CD25 can be used to distinguish neoplastic from reactive mast cells in GI biopsies.
Design: 9 GI biopsies from 5 patients with SM, 13 GI biopsies from 5 patients with UP, and 50 control cases including 5 each H. pylori gastritis, bile reflux gastropathy, peptic duodenitis, celiac disease, Crohn's disease, ulcerative colitis, lymphocytic colitis, collagenous colitis, parasitic infections, and eosinophilic gastroenteritis were immunostained for mast cell tryptase, c-kit (CD117), and CD25. Mucosal mast cells were quantitated, and the presence or absence of CD25 expression on mast cells was determined.
Results: In SM patients, mast cells numbered >100/hpf in all cases (mean 178/hpf; range 110-301). This was significantly higher than in GI biopsies from UP patients (mean 17/hpf; range 8-32, $p<0.001$) and all inflammatory diseases ($p<0.05$). Mast cell density in other disorders ranged from a mean of 6/hpf in H. pylori gastritis to 39/hpf in parasitic infections. Interestingly, all SM biopsies (and none of the other cases) contained aggregates or confluent sheets of mast cells. In addition, mast cells in all SM cases were positive for CD25, whereas GI mast cells in UP and all other control cases were negative.
Conclusions: Quantitation of mast cells can be helpful to diagnose SM in GI biopsies, although mast cells are also markedly increased in parasitic infections. Aggregates or sheets of mast cells are only seen in SM. Immunoreactivity for CD25 in GI mucosal mast cells is specific for SM and can be used to confirm the diagnosis.

522 Immunostains for CDX-2 and Monoclonal CEA Can Distinguish Crushed Benign Gastric Cells and Signet Ring Cell Carcinoma

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Background: Gastric biopsies often have focal crush artifact that disperses the gastric epithelium into single cells with signet ring-like features, and can be mistaken for signet ring cell carcinoma, particularly when the biopsy specimens are small. We investigated the utility of two markers, monoclonal CEA (mCEA), which is expressed in gastrointestinal epithelial cells, and cdx-2, which is expressed only in gastric carcinoma/dysplasia but not in normal gastric epithelium, to differentiate crushed benign epithelium from gastric carcinoma.
Design: Gastric biopsies from eighteen cases with a diagnosis of poorly differentiated gastric carcinoma with prominent signet ring features, and eleven benign stomach biopsies with crushed epithelium and signet ring-like cells were included in this study. Immunohistochemistry for cdx-2, mCEA, and cytokeratin AE1/3 was performed on each biopsy.
Results: Single cells with signet ring features were present in both groups of cases. The majority of the carcinoma cases showed mild to moderate cytologic atypia. Crush artifact obscured the cytologic features in the benign mucosa. Immunostain for AE1/2 highlighted single cells in 100% (18/18) of the carcinoma cases and 91% (10/11) of the benign biopsies with similar intensity. Therefore, a distinction based on histologic appearance and AE1/3 immunohistochemical stain was not reliable. In the carcinoma group, signet ring cells were positive for cdx-2 in 16 of 18 (89%) cases, and were positive for mCEA in 15 of 18 (83%) cases. Therefore, all of the carcinoma cases were positive for either cdx-2 or mCEA, or both. In contrast, the signet ring-like single cells were negative for both cdx-2 (0/11) and mCEA (0/11) in all of the benign gastric biopsies.
Conclusions: Crush artifact can sometimes pose a significant challenge in the evaluation of gastric biopsies, as crushed benign epithelial cells can resemble and be mistaken for signet ring cell carcinoma. Our study showed that immunohistochemical stains for mCEA and cdx-2 can help distinguish between these two entities, and can be used as a reliable ancillary tool in these potentially difficult cases.

523 Progressive Increase in PPM1D/wip-1 Protein Expression in Normal Mucosa, Tubular Adenoma, and Low- and High-Grade Colorectal Adenocarcinoma

DP Hartmann, BVS Kallakury, J Luka, EA Apella, N Azumi. Georgetown University Hospital, Washington, DC; Bioworld Consulting Labs, New Windsor, MD; NIH, Bethesda, MD.
Background: The protein encoded by the PPM1D/wip-1 gene is a member of the PP2C family of Ser/Thr protein phosphatase which down-regulates the apoptotic activity of p53. We have previously reported the correlation of wip-1 over-expression with higher stage, higher grade and the presence of lymph node metastasis in colorectal carcinoma.
Design: Using a novel monoclonal antibody to wip-1(Bioworld), a tissue microarray of formalin-fixed tissue consisting of 34 normal colonic mucosa, 26 tubular adenomas, 23 low-grade and 19 high-grade colorectal adenocarcinomas was tested for wip-1 expression immunohistochemically to further elucidate the correlation of protein expression and progression of colorectal carcinogenesis. Staining was scored on a scale of 0-3+, based on intensity and percentage of positive cells.
Results: Immunoreactivity for wip-1 protein was exclusively intranuclear and was seen in 4/34 (12%) normal mucosa, 26/26 (100%) adenomas and 42/42 (100%) carcinomas . However, only 6/26 adenomas (23%) showed 2+ as compared to 34/42 (81%) carcinomas showed 2+ or 3+. There was no statistical difference in wip-1 positvity between low and high grade carcinomas.

Tubular Adenomas

Staining	0	1+	2+	3+
Sample#	0	20	6	0

Adenocarcinomas

Staining	0	1+	2+	3+
Sample#	0	8	31	3

Conclusions: Expression of wip-1 progressively increases from normal mucosa to neoplastic lesions suggesting its role in the carcinogenesis. wip-1 appears as early carcinogenic event in formation of adenomas and, then, the expression intensifies as adenomas progress to carcinomas. These results also support wip-1 as a potential therapeutic target in the treatment of carcinomas of colon and other organs.

524 High Frequency of Platelet Derived Growth Factor Receptor Alpha Mutations in Gastrointestinal Stromal Tumors: Detection by High Resolution Melting Amplicon Analysis

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Background: Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumor of the gastrointestinal tract. Approximately 2-3000 new cases are diagnosed each year. GIST result from mutation in either the c-kit gene or the platelet derived growth factor receptor alpha (PDGFRA) gene. Activated KIT proteins are inhibited by imatinib but GIST with actived PDGFRA are relatively resistant. Because GIST with different activating mutations behave differently in response to imatinib therapy, it is useful to determine the c-kit and PDGFRA mutation status in GIST prior to therapy. We are using high resolution DNA melting curve analysis to routinely provide this molecular information. In reviewing our series of 83 cases, we found that PDGFRA activating mutations make up almost 1 in 5 GIST, higher than previously reported. In addition we found one PDGFRA mutation that has not yet been described.
Design: A total of 83 GIST were evalutated. Sixty-five were from patients at the University of Utah Health Sciences Center and the H. Lee Moffitt Cancer and Research Institute. Eighteen cases were referred from outside institutions. The diagnosis of GIST was based on the morphologic pattern of the tumor, a positive KIT immunostain or a positive PDGFRA mutation result. Antibodies against KIT (CD 117) were used at a dilution of 1:400 without antigen retrieval. DNA was isolated from paraffin embedded tissue sections and evaluated by melting curve analysis for activating mutations in c-kit exons 9,11,13, and 17 and for PDGFRA mutations in exons 12 and 18.
Results: Activating mutations were detected in 76 of 83 (92%) GIST. Of the 76 mutation positive GIST, 60 (79%) contained c-kit mutations and 16 (21%) contained PDGFRA mutations. One PDGFRA mutation postive case had a mutation (D842V del 843) that has not been described.
Conclusions: Melting curve anlysis was able to detect c-kit or PDGFRA activating mutations in 92% of 83 cases diagnosed as GIST. Surprisingly, we found that PDGFRA mutation positive GIST made up about 1 in 5 of our cases. Because PDGFRA mutation positive GIST may result in a relatively imatinib resistant protein, it is important to detect this molecular alteration.

525 Expression of Ductular Profile Cytokeratins in Hepatocellular Carcinoma with and without Cirrhosis

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Background: Although cirrhosis plays an important role in liver carcinogenesis, about 15% of hepatocellular carcinoma (HCC) arises from non-cirrhotic liver. A significant survival advantage in HCC without cirrhosis over HCC with cirrhosis has been well documented. However, the underlying molecular mechanisms responsible for the clinical differences between HCC with and without cirrhosis is uncertain. This study addresses the potential role of ductular differentiation in liver carcinogenesis of these two types of HCC and its clinical implications.
Design: We studied the clinicopathology and the immunohistochemical expression of CK7 and CK19 in a tissue microarray composed of 20 normal livers, 20 cirrhotic livers, tumors and their adjacent non-neoplastic background liver tissue in 20 HCCs with cirrhosis and 20 HCCs without cirrhosis.
Results: Cirrhotic liver showed a statistically significant higher level of CK7 and CK19 expression than normal liver. HCC with cirrhosis showed a statistically significant higher level of CK7 and a trend toward higher CK19 expression than HCC without cirrhosis. A statistically larger proportion of HCC patients with viral etiology (6/16) expressed CK7 and CK19 than HCC patients with alcoholic steatohepatitis (0/7) and HCC patients without well-defined underlying liver diseases (2/13). CK7 and CK19 expression was more commonly seen in WHO grade II and grade III tumors than grade I tumors (9/36 vs. 1/36 for CK7, 2/36 vs. 0/36 for CK19). Patients with HCC without biliary profile cytokeratin expression showed a significantly longer disease free survival than patients with HCC with biliary profile cytokeratin expression ($P<0.05$, Log rank test).
Conclusions: The higher level of biliary profile cytokeratin expression in the tumor tissue of HCC with cirrhosis and cirrhotic liver are most likely due to a persistent process of hepatocyte regeneration, hepatocyte precursor cell activation, and ductular proliferation in response to persistent hepatocyte damage, particularly from viral hepatitis. Combined analysis of our data and previously reported studies suggest two different types of HCC: one originating from hepatocyte progenitor cells, characterized by viral etiology, cirrhosis, biliary cytokeratin expression, and poorer survival; the other originating from mature hepatocytes, characterized by non-viral etiology, without cirrhosis, without biliary cytokeratin expression, and longer survival.

526 Biological Properties of Buried Intestinal Epithelium Following Photodynamic Therapy for Barrett's Esophagus

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Background: Photodynamic therapy (PDT) is increasingly used for the treatment of patients with Barrett's esophagus (BE). Post PDT, most BE patients develop islands of re-epithelialized squamous mucosa overlying buried Barrett's epithelium (BBE). A previous study by our group showed that BBE in BE patients treated with proton pump inhibitors (PPI) shows a significantly lower Ki-67 proliferation rate compared to non-buried BE. However, the biological properties of BBE post PDT, an epithelium commonly believed to have increased neoplastic potential, have not been studied. Therefore, the aim of this study was to evaluate the biological characteristics of BBE in BE patients treated with PDT.

Design: 24 non-dysplastic esophageal mucosal biopsies from 12 BE patients (M/F ratio: 9/3, mean age: 68 yr, mean length of BE: 8.8 cm) with BBE (mean: 2 biopsies per patient, range: 1-7) following PDT (mean interval: 13 months, range 3-38 months) were immunostained for Ki-67, p53, cyclin D1, bcl-2, TGF-α, EGFR, and AMACR and evaluated for the crypt proliferation index and the presence and degree of staining. The findings in BBE were compared to comparable levels of crypts in adjacent areas of non-buried BE.

Results: BBE showed a significantly lower Ki-67 crypt proliferation rate (29.9% vs. 44.4%, $P<0.001$), in comparison to non-buried crypts in adjacent areas of BE. BBE and adjacent BE showed a similar rate of positivity, and location of staining, for p53 (13% vs. 15%), cyclin D1 (4% vs. 5%), bcl-2 (17% vs. 15%), TGF-α (63% vs. 80%), EGFR (33% vs. 35%), and AMACR (0% vs. 0%). Interestingly, the Ki-67 proliferation rate in BBE post-PDT was similar to our previously published value for BBE post-PPI therapy (28.6%), the latter of which was evaluated utilizing the same methodology.

Conclusions: Similar to PPI-treated patients, BBE post-PDT shows a decrease in crypt proliferation, but otherwise similar biological properties, compared to non-buried Barrett's epithelium. Reduced proliferation in BBE may be due to a decrease in exposure to the trophic effects of luminal contents as a result of protective overlying squamous epithelium. BBE post-PDT may not be at increased risk for neoplastic transformation above that of the non-buried BE.

527 Application of Microarray-Based CGH Technology To Identify a Novel Recurrent Gene Dosage Alteration Common to Colorectal Carcinomas and Adenomas

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Background: Advanced colorectal cancer (CRC) often show large segmental chromosomal alterations by conventional comparative genomic hybridization (cCGH) including recurrent alterations in 13q and 18q. The clonal nature would suggest a proliferative advantage or an apoptotic disadvantage in CRC development. Sensitive microarray measures of gene dosage are best performed using optimal DNA template from fresh frozen tissue samples, but clinical outcomes are limited.

Design: We have developed protocols for DNA extraction and processing using formalin fixed paraffin embedded (FFPE) tissue sections for downstream genomic analyses including microarray CGH. The Agilent 44 K feature 60-mer oligonucleotide microarray for CGH was used to hybridize 14 advanced CRC and 10 colorectal adenomas. FFPE DNA showed variable fragment length in the 500 base pair to 12 kb range by Sybr-Gold gel electrophoresis. To generate more uniform DNA template, the sample and sex-mismatched genomic reference DNA samples were DNase digested prior to hybrdization. CGH Analytics 3.324 software and quantitative algorithm of ADM, Agilent Inc, Palo Alto, CA provide data visualization and analyses.

Results: The majority of the CRC samples showed significant genomic instability with frequent alterations of entire chromosome arms; however, several CRC samples showed remarkably stable genomes. One such stable genome CRC showed a discrete gain spanning 150 kilobase on 7p that is annotated to be a gene family critical in embryological gastrulation in a specific temporal-spatio pattern. Stringent algorithm criteria identified this alteration in 5 of 14 CRCs and an additional 4 CRCs showed significant increase in hybe signal over baseline. Hybridization of 9 advanced adenomas(6 by villous morphology and 3 by size criteria) and 1 tubular adenoma (7mm) , showed more stable genomes with occasional partial loss of 18q present. 5 of 9 advanced adenomas and the non-advanced adenoma showed the same discrete 7p alteration noted in the CRCs. Hybridization data on 7 additional non-advanced adenomas will be presented.

Conclusions: Here we demonstrate that sensitive and specific gene dosage measures by array CGH detects a novel recurrent gene dosage alterations common to colorectal adenomas and carcinomas. Further functional characterization will be performed by immunohistochemistry on tissue microarrays.

528 Importance of Elastic Stains To Demonstrate Venous Invasion in Colorectal Carcinomas

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Background: Large vessel (venous) invasion (VI) is an important prognostic factor in colorectal carcinoma (CRC) due to the positive association with liver metastases. Both extramural and intramural VI are independent prognostic factors in CRC. Our objectives were to determine the importance of using an elastic stain to facilitate diagnosis of VI when compared to hematoxylin and eosin (H&E) stained slides only.

Design: CRC cases from the year 2000 (n=92) were randomly selected from departmental files. The H&E-stained slides containing tumor were reviewed by one GI pathologist, one non-GI pathologist, and one pathology resident, and categorized as positive, negative or equivocal for VI. Each investigator then selected one block from each case that was considered most likely to contain VI. Movat pentachrome stains were performed on all tumor blocks and evaluated as positive or negative for VI. These results were analyzed to determine whether Movat stains on all blocks increased the yield of detection compared with stains on a single block. Statistical analysis was performed with the χ^2 test.

Results: Of 92 CRC cases, 84% were adenocarcinoma nos, 16% were mucinous carcinoma; 12% were stage T2 and 88% were T3/T4. 62% had VI (56% extramural, 25% intramural, 19% both extra- and intramural) after evaluation of all Movat stained sections. Evaluation of the H&E slides alone showed that 17 (18%) were positive, 50 (54%) were negative and 25 (27%) were equivocal for VI, after grouping the investigators' independent findings. Sixteen of the positive group (94%), 19 of the equivocal group (76%) and 22 of the negative group (44%, $p<0.001$) showed VI using the elastic stain. Movat stains on all blocks increased the overall yield of detection from 52% to 62% when compared with stains on a single block (p=ns)(grouped data). Extramural VI was identified in the majority of cases as round to oval foci adjacent to muscular arteries, which protrude downward in tongues from the deepest edge of the tumor into pericolic/rectal fat.

Conclusions: The detection of venous invasion is significantly aided by the use of an elastic stain. The added yield of VI detection in H&E-negative cases using Movat stains on one vs. all tumor blocks was not significant. We recommend that at least one tumor section should be selected for an elastic stain in all CRC cases thought to be negative or equivocal for venous invasion on H&E stains; blocks that contain suspicious foci (as noted above) should be targeted.

529 Distinct CK7 Staining Pattern in Focal Nodular Hyperplasia and Hepatic Adenoma and Its Diagnostic Significance

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Background: Focal nodular hyperplasia (FNH) is a benign hyperplastic lesion that is often managed conservatively, while hepatic adenoma (HA) is a neoplastic lesion that is commonly resected. While it is important to differentiate these two lesions, it is often difficult to make this distinction on a core needle biopsy specimen. CK7 in liver is expressed in biliary epithelium, canals of Herring and probably hepatic stem cells. The purpose of this study was to examine the expression pattern of CK7 immunostain in FNH and HA, and assess its diagnostic utility.

Design: Resection specimens of HA (n=5) and FNH (n=5) and telangiectatic FNH (n=1) were selected for the study. The hematoxylin and eosin-stained sections in all cases were reviewed and CK7 immunostain was performed on formalin-fixed, paraffin-embedded representative sections from each case.The CK7 staining intensity and pattern were analyzed in each case.

Results: In every case of the normal non-lesional liver (n=11), the CK7 immunostain strongly stained the bile ducts and ductules and acted as controls. Mild and focal CK7 staining was seen in the periportal hepatocytes of normal liver in one case only. In the HA specimens, there was patchy moderate to strong CK7 staining of the hepatocytes, most notable adjacent to intra-lesional fibrovascular septae. Although, bile ducts were typically absent in HA, occasional ductules could be identified with CK7 stain. In the FNH specimens, CK7 strongly stained the bile ducts and ductules in the fibrous septae. The CK7 staining in the hepatocytes was seen in the periphery of most lobules and was milder and focal compared to HA in all cases, except one case which also showed patchy HA-like staining pattern. The case of telangiectatic FNH showed both HA-like and FNH-like CK7 staining pattern in different areas of the lesion.

Conclusions: Our study shows that the staining pattern of CK7 in HA and FNH is distinct and can be diagnostically useful. The CK7 staining pattern could also help in differentiating normal and lesional tissue in needle biopsies, particularly in HA cases. The CK7 staining pattern in telangiectatic FNH also supports the view that these lesions are similar to HA. The variations in CK7 staining in hepatocytes possibly represent varying proliferative patterns in different lesions.

530 Age-Associated Immunosenescence Is a Major and Unrecognized Risk Factor for Herpes Simplex Virus Esophagitis

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Background: All three most common esophagitis-causing infectious agents, herpes simplex virus (HSV), cytomegalovirus (CMV) and candida are known to affect immunocompromised patients. While therapy and disease related acquired immunodeficiencies are recognized risk factors in these infections, many cases of HSV esophagitis occur in seemingly immunocompetent patients over the age of 65. In these older patients, natural age-associated immunosenescence appears to be the major and unrecognized risk factor.

Design: 21 patients (12 males and 9 females, age range 31-89 years) were diagnosed with HSV esophagitis on esophageal biopsies in our institution in the last 7 years. Their biopsies and clinical, laboratory and therapeutic histories were reviewed. These patients were separated in two sets of groups: patients over 65 (>65) and under 65 (<65) and patients with and without identifiable acquired immunodeficiencies.

Results: All patients had unequivocal HSV esophagitis with characteristic herpetic inclusions. 57% (12/21) of patients were >65 and 33% (7/21) were over 75 years of age. Only 25% of >65 patients (4/12) showed identifiable acquired immunodeficiencies (prostate carcinoma, CLL and liver transplantation with immunosuppression), while 100% cases <65 (9/9) had clearly identified immunodeficiencies such as HIV (3/9), acute leukemia (2/9), metastatic colon and renal carcinoma with chemotherapy (2/9) and renal and liver transplantation-related immunosuppression (2/9). The majority (75%) of >65 patients had no identifiable immunodeficiency other than natural age-related immunosenescence.

Conclusions: Age-related immunosenescence is an unrecognized and a major risk factor for HSV esophagitis. The majority of HSV esophagitis patients are older than 65, and many of these have no identifiable immunodeficiency other than age-associated immunosenescence. The central role of immunodeficiency in the causation of HSV esophagitis is exemplified by otherwise healthy <65 patients affected only when they have therapy and/or disease related acquired immunodeficiencies.

531 Expression of Phosphorylated Akt Kinase (p-Akt), Phosphorylated Mammalian Target of Rapamycin (p-mTOR), and Phosphorylated p70 S6 Kinase (p-p70S6K) in Colorectal Adenocarcinoma (CRC)

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Background: Akt (protein kinase B), mTOR, and p70S6 kinase are members of the phosphatidylinositol 3 kinase (PI3K) signaling pathway that regulates cell growth and impacts other growth factors, mitogens, cell size and overall cellular homeostasis. Although the PIK3 pathway has been studied in a variety of human neoplasms, the potential roles of Akt, mTOR, and p70S6 in colorectal cancer tumorigenesis and prognosis has not been previously studied.

Design: Formalin-fixed, paraffin-embedded sections from 108 CRC were immunostained with an automated method (Ventana, Tuscon, AZ) using antibodies (Cell Signaling, Danvers, MA) against p-Akt, p-mTOR, and p-p70S6K. Expression was semiquantitatively evaluated for both tumor and adjacent benign mucosa based on intensity and distribution using a three-tiered system (weak/focal 1-25%, moderate/regional 25-75% and strong/diffuse 75-100%).

Results: Tumor immunoreactivity ranged from over-expression, similar to and under-expression in relation to the benign mucosa for all proteins. Overall, 88%, 78%, and 59% tumors featured membranous/cytoplasmic over-expression for p-Akt, p-mTOR and p-p70S6 respectively; 2%, 4%, and 16% were similar to, and 10%, 18%, and 25% under-expressed. Tumor cell membrane p-mTOR over-expression correlated with high tumor grade (80% Grade 3 vs 50% Grade 1 p=0.042) and age at diagnosis (79% oldest group vs 0% youngest group; p=0.007), while tumor cell cytoplasmic over-expression for p-mTOR correlated with older age (79% oldest group vs 0% youngest group; p=0.002). A trend towards over-expression of p-p70S6 was observed in high tumor grade (80% grade 3 vs 62% grade 2 vs 20% grade 1; p=0.076) was observed. p-p70S6 cytoplasmic over-expression correlated with mucin-deficient tumor cells (70% mucin deficient vs 49% mucin-rich; p=0.004). Akt, mTOR, and p70S6 expression did not correlate with tumor stage, lymph node status, sex, Crohn's disease status, or survival.

Conclusions: p-AKT, p-mTOR and p-p70S6 are over-expressed in CRC vs adjacent benign mucosa. p-mTOR overexpression correlates with high tumor grade and older age at diagnosis and p-p70S6 overexpression correlates with mucin-deficient tumors. These observations indicate that PI3K pathway members play significant roles in CRC tumorigenesis and warrant further study as potential prognostic factors.

532 Adult-Onset Autoimmune Enteropathy

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Background: Autoimmune enteropathy (AIE) is a rare disorder characterized by intractable diarrhea and circulating autoantibodies against enterocytes or goblet cells. The disease primarily affects children, with only rare reported cases of adult-onset AIE. In this study, we systematically describe a series of adult-onset AIE patients from a single institutional experience.

Design: The study included 10 adults who were clinically diagnosed with AIE from 2001-2006. Clinical data were abstracted from their computerized records. We reviewed duodenal/jejunal biopsies from all patients and, where available, biopsies from stomach and colon. The following features were scored: 1) villous/crypt (v/c) ratio, 2) surface intraepithelial lymphocytosis, 3) crypt lymphocytosis, 4) crypt apoptoses, 5) basal plasmacytosis, 6) neutrophilic inflammation, and 7) absence of goblet and Paneth cells.

Results: There was no gender predilection in adult AIE (5M/5F). Mean age was 52 yrs (range 36-76 yrs). All suffered from prolonged diarrhea without response to gluten-free diet. Nine (90%) had positive anti-enterocyte (n=7) and/or anti-goblet cell (n=4) antibodies; in one patient with immunodeficiency the serology was negative, but he responded to immunosuppression and relapsed when steroids were withdrawn. Histologically, 8 (80%) showed features typical of AIE described in children, including deep crypt apoptoses (mean 1.03/crypt, range 0.5-3), variable villous blunting (mean v/c ratio 1/2.2, range 2/1-complete blunting), increased deep crypt lymphocytosis (mild in 7, minimal in 1, lymphoepithelial lesions in 3), basal plasmacytosis (marked in 4, mild in 2), and neutrophilic inflammation (crypt abscesses in 2, cryptitis in 3). Goblet cells were completely absent in 5 cases and Paneth cells in 3. In contrast to celiac disease, these 8 patients showed no excess surface lymphocytosis (n=6) or only borderline lymphocytosis (n=2, both with 30 lymphocytes:100 enterocytes). The other 2 patients displayed histologic features of both AIE and celiac disease, with moderate neutrophilic inflammation, absent goblet cells in one, and increased surface intraepithelial lymphocytosis (50 and >100 lymphocytes:100 enterocytes); one had positive celiac serologies. Concomitant biopsies from colon and stomach showed autoimmune colopathy in 4 of 10 (40%) cases and autoimmune gastritis in 2 of 6 (33%).

Conclusions: Histologic features of adult AIE are similar to those described in children, but overlap with celiac disease can exist. AIE is rare in adults, but it may be underdiagnosed. Correlation of the histology and serologies for AIE and celiac disease is essential.

533 Lymphovascular Invasion in Colorectal Cancer: An Interobserver Variability Study

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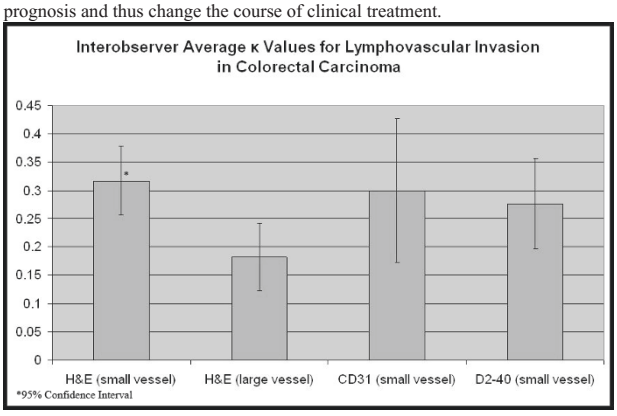
Background: Lymphovascular invasion (LVI) in colorectal cancer (CRC) is considered a strong stage-independent prognostic factor and influences decisions regarding adjuvant chemotherapy in patients with Stage II tumors. This study was undertaken to test the hypothesis that interobserver agreement among pathologists for LVI in CRC is relatively poor, but might be improved with use of lymphvascular immunohistochemical markers.

Design: Fifty cases of AJCC stage II moderately differentiated CRC from 1990 to 2005 from the pathology archives were selected, excluding mucinous and medullary subtypes. Fifty H&E slides (one from each case) were circulated to 6 GI pathologists, who independently assessed small and large vessel invasion. No diagnostic guidelines were given to the participating pathologists. Immunohistochemistry (IHC) for D2-40 and CD31 was performed on corresponding paraffin blocks. The IHC slides were randomized, recirculated, and rescored for LVI. Results were analyzed by κ statistics, which correct for agreement by chance.

Results: The average κ values were determined for the H&E slides (large and small vessel), CD31 (small vessel), and D2-40 (small vessel) (Figure 1). Agreement was fair for H&E small vessel invasion (κ = 0.32; 95%CI 0.26-0.38). The least agreement was seen in interpretation of H&E large vessel invasion (κ = 0.18; 95%CI 0.12-0.24). Agreement was not improved by use of immunohistochemical stains: CD31 (κ = 0.30, 95%CI 0.17-0.43) and D2-40 (κ = 0.28, 95%CI 0.20-0.36).

Conclusions: Interobserver variability in diagnosis of LVI was substantial on H&E slides and did not improve upon use of IHC. Agreement in evaluation of large vessel invasion was only slightly higher than would be seen by chance alone. This study highlights the need for criteria in assessment of lymphovascular invasion, as this may impact patient prognosis and thus change the course of clinical treatment.



534 Fatty Acid Synthase Overexpression in Colorectal Cancer Is Associated with Microsatellite Instability, Independent of CpG Island Methylator Phenotype (CIMP)

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Background: Overexpression of fatty acid synthase (FASN), a key enzyme for de novo lipogenesis, is observed in many cancers including colorectal cancer, and is associated with poor clinical outcomes. Energy excess physiologically upregulates cellular FASN expression level. Obesity and excess energy balance have been known to be risk factors for colorectal cancer. Microsatellite instability-high (MSI-H) is a distinct phenotype in colorectal cancer, associated with a variety of pathologic and molecular features, including CpG island methylator phenotype (CIMP). Previous data suggest that obesity or altered energy balance may potentially modify risks for MSI-H cancers and microsatellite stable (MSS) cancers differently. However, relationship between MSI and FASN overexpression has not been investigated.

Design: Utilizing 976 cases of population-based colorectal cancer samples from two large prospective cohort studies, we correlated FASN expression (by immunohistochemistry) with MSI, *KRAS* and *BRAF* mutations, p53 expression (by immunohistochemistry), pathologic features and CIMP status [determined by quantitative MethyLight assays for eight CIMP-specific gene promoters including *CACNA1G*, *CDKN2A* (p16), *CRABP1, IGF2*, *MLH1*, *NEUROG1, RUNX3* and *SOCS1*]. CIMP-high was defined as ≥6/8 methylated promoters, based on *KRAS* and *BRAF* mutation rates.

Results: Marked (2+) FASN overexpression was observed in 110 (11%) of the 976 tumors, and was significantly more common in MSI-H tumors (21%=28/135) than MSI-low (5.6%=4/72, p=0.004) and MSS tumors (11%=72/678, p=0.001). Although FASN overexpression appeared to be more common in CIMP-high tumors (17%, p=0.05) than non-CIMP-high tumors (11%), FASN expression was no longer significantly related to CIMP after stratification by MSI status. In contrast, the association between FASN overexpression and MSI-H persisted even after stratification by CIMP status. FASN overexpression was also significantly correlated with the presence of mucinous component, Crohn's-like reaction, tumor infiltrating lymphocytes and expansile tumor border (all p<0.03). FASN overexpression was not significantly related with sex, tumor location, p53, or *KRAS*/*BRAF* status.

Conclusions: FASN overexpression in colorectal cancer is associated with MSI-H independent of CIMP status. Our data suggest a possible pathogenetic link between MSI and FASN overexpression.

535 Pitfalls in the Staging of Ampullary Carcinomas: Is the Current TNM (AJCC/UICC) System Pathologically and Clinically Relevant?

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Background: The ampulla of Vater is a complex region, and the tumors of this region are relatively rare and thus less carefully scrutinized. Consequently, the applicability and clinical validity of the current staging system has not been verified.

Design: AJCC TNM staging parameters were tested and correlated with the clinical outcome in primary ampullary carcinomas. A carcinoma was classified as *ampullary* only if its epicenter was in the ampulla of Vater (and/or major papilla), and if a pre-invasive tumor was present in the ampullary (or papilla) mucosa.

Results: Among 483 cases of pancreato-duodenal resections for adenoca., 45 cases fulfilled these criteria of ampullary ca. 3 components of the staging parameters were found to be problematic: 1) It was not clear whether "invasion to duodenum" implied involvement of any layer of duodenum including the mucosa. Due to the indistinct nature of the transition from Vaterian to duodenal mucosa, most (91 %) of the cases analyzed had some degree of duodenal mucosal/submucosal involvement (T1 vs T2). 2). It was difficult to determine whether a tumor invading through the sphincter (and duodenal musculature) into the subserosa and wrapping around the pancreas at the "groove" area was to be regarded as "extrapancreatic soft tissue extension" (T2 vs T3 vs T4), which was an issue in 37 % of the cases. 3) In 2 cases (5%), there was involvement of scattered pancreatic acini without any distinct lobule formation was (heterotopia possible;T3 vs T4). When duodenal staging system was applied, it was found to be a better indicator of 5-year survival (corr. coef. -0.523, P=0.001) then the ampullary one (corr. coef. -0.358, P=0.024). Size of invasive ca. was not a good indicator of survival (corr. coef. -0.050, P=0.756).

Conclusions: Current TNM staging guidelines for ampullary tumors need substantial clarification due to lack of histopathologic applicability. Staging principles applied on the intestines, by taking the ampulla as a "tubular organ" and regarding it as in continuum with duodenum, appears to be a better indicator of clinical outcome. The lack of correlation with size of invasive carcinoma also supports the use of tumor depth rather than the involvement of adjacent organs.

536 Methylation Profiles of Multiple CpG Island Loci in Extrahepatic Cholangiocarcinoma Versus Those of Intrahepatic Cholangiocarcinomas

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Background: Extrahepatic cholangiocarcinoma (ECC) has a poor prognosis, which is largely attributed to an advanced disease stage at presentation. Thus, early detection and curative resection would improve its outcome. CpG island hypermethylation is attracting attention due to its importance as a tumor marker as well as potential mechanism of the development of human cancers.

Design: In the present study, the prevalence of hypermethylation in a panel of 24 CpG island loci was determined in an ECC series and compared with that of intrahepatic cholangiocarcinoma (ICC). Tumor samples from 63 ECCs and 48 ICC were investigated for hypermethylation in 24 CpG island loci using methylation-specific PCR.

Results: A total of 96.8% (61 of 63) of ECCs showed hypermethylation in at least one of the examined loci. In ECC, a high frequency of methylation was seen in *HOXA1* (95.2%), *HPP1* (69.8%), *NEUROG1* (61.9%), *CDH1* (39.7%), MINT1 (36.5%), and *RUNX3* (30.2%). Papillary gross type ECC displayed a higher number of methylated loci than nodular or diffuse gross types ($p<0.05$). *CDH1* and *NEUROG1* were more frequently methylated in ECC than in ICC, whereas *CHFR, GSTP1, IGF2, MGMT*, MINT31, *p14*, and *RBP1* were more frequently methylated in ICC ($p<0.05$).

Conclusions: Our findings show that CpG island hypermethylation is a frequent finding in ECC and ICC, and that ICC and ECC exhibit shared but distinct methylation profiles. *HOXA1* was methylated in the vast majority of ECC, and we suggest that it might serve as a potential molecular marker for the detection of ECC, especially in bile juice.

537 Methylation Profile of the Promoter CpG Islands of 40 Genes in Colorectal Cancer and Its Relationship to Clinical Features

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Background: Abberant methylation of promoter CpG island is associated with gene silencing and may lead to carcinogenesis. The aims of our study were to investigate the methylation profile of multiple genes in colorectal cancer (CRC) and to correlate the findings with clinical features-patient age, gender, survival, tumor location and microsatellite instability (MSI) status.

Design: A total of 140 CRCs and their corresponding normal mucosa tissue specimens were examined for the methylation status of 40 genes using methylation-specific PCR. These findings were analyzed for the correlation of methylation status of genes with each of clinical features.

Results: Ninety percent of the 40 genes showed hypermethylation more than 10% in CRC and all the cases exhibited hypermethylation of at least two genes (ranging from 2 to 34 genes). Seventy-nine percent of the tested genes were methylated in cancer more frequently than in corresponding normal tissue with statistically significant difference. An association of hypermethylation with older age (age≥62) was found in five genes, while one gene showed an association of hypermethylation with younger age (age<62). Although older age group showed higher number of genes methylated than younger age group, the difference was not significant. Three genes exhibited significantly higher frequency of methylation in female patients but the mean number of methylated genes did not show a significant difference between male and female patients. Hypermethylation of 11 genes was closely associated with right localization, whereas one gene with left-sided CRC. MSI-positive cancer, compared with MSI-negative cancer, has significantly higher frequency of methylation in 12 genes. Multivariate analysis revealed that hypermethylation of four genes (CDH1, HPP1, IGF2 and NR3C1) was closely associated with patient survival.

Conclusions: We produced methylation profile of 40 genes in 140 CRCs and found that variable relationship between hypermethylation and clinical features existed depending on the type of gene. Notably, MSI-positive CRC and right-sided CRC tend to exhibit higher frequency of methylation. CpG island hypermethylation of four genes exhibited a promising prognostic value but should be subjected to a large scaled further study.

538 Systemic Mastocytosis Involving the Gastrointestinal Tract: A Clinicopathologic Study of 5 Cases

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Background: Systemic mastocytosis (SM) rarely involves the gastrointestinal (GI) tract and only a few cases reported in the literature. We report a series of five cases and describe the spectrum of clinicopathologic features.

Design: Five cases of SM were retrieved from the consultation files of 4 GI pathologists (R.H.R., K.G., G.D.H., N.S.). Where possible, clinical details were obtained from the hospital of origin or referring pathologist. Histologic features were documented in slides stained with HE, mast cell tryptase and CD117.

Results: The age range was 12-55yr (median 38yr). All patients were female. The duration of symptoms was 0.5-5 yrs. Symptoms included diarrhea/loose stools (n=5), vomiting (n=3), loss of weight (n=3) and cramping abdominal pain (n=2). Cutaneous lesions of mastocytosis were documented in 4 patients, hypoalbuminemia in 2 and constitutional growth delay in 1 patient. Two patients presented with de novo GI involvement (i.e. preceding other lesions of SM). The sites of GI involvement included the colon (n=5), duodenum (n=2) and terminal ileum (n=2). Endoscopic/gross findings included mucosal multinodularity (n=3), erosions (n=2), loss of mucosal folds (n=2). In three patients the endoscopic appearance was that of inflammatory bowel disease (IBD), and two were treated as such. Histologic findings included mast (MC) cell infiltration of the lamina propria (n=5), which was diffuse in 4 cases. In 2 cases, MC had abundant clear cytoplasm and were initially thought to be histiocytes. A florid eosinophil infiltrate was present in 4 patients with prominent immature forms in two, leading to an initial diagnosis of "eosinophilic colitis" in one patient. Mild to moderate architectural distortion was noted in 3 cases. Immunohistochemistry for MC tryptase and CD117 confirmed the MC infiltrate in all cases. Clinical follow up is limited as most cases were recently diagnosed.

Conclusions: 1) SM involvement of the GI tract is characterized by a spectrum of morphologic features that can be mistaken for IBD, eosinophilic infiltrate (colitis) or histiocytic infiltrate. 2) An awareness of this condition is important to facilitate accurate diagnosis and timely institution of appropriate therapy.

539 Expression of CK7, CK20, CDX2, and CA19.9 in Pancreatic Endocrine Neoplasms of the Pancreas (PENs): A Tissue Microarray Analysis

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Background: Distinguishing pancreatic endocrine neoplasms (PENs) from other pancreatic neoplasms based on morphology alone is not always straightforward, particularly on needle biopsy. The histologic appearance of PENs varies significantly, ranging from PENs with classic nested pattern and "salt-and-pepper" chromatin to PENs resembling adenocarcinoma with acinar structures composed of cells with large vesicular nuclei and prominent nucleoli. Limited studies have suggested that PENs can share immunohistochemical patterns with ductal tumors of the pancreas. This overlap of morphology and immunohistochemical expression may lead to misdiagnosis. The purpose of this study was to determine immunohistochemical patterns of PENs based on their expression of CK7, CK20, CDX-2, and CA 19.9.

Design: Tissue cores were collected from routinely processed paraffin-embedded tissue of 45 PENs, all confirmed to be chromogranin-positive. From each tumor case 5 tissue cores, each 2 mm in diameter, were taken from random areas of the tumor. The tissue cores were then arranged in tissue microarrays (TMAs) and immunohistochemically stained for CK7, CK20, CDX-2 and CA 19.9. The degree of staining was graded as 1+ (1% to 10%), 2+ (11% to 25%), 3+ (26% to 50%) and 4+ (>50%).

Results: Eight of 45 PENs (18%) were positive for CK7 (four 1+, four 4+). Four of 45 (9%) were positive for CK20 (three 1+, one 2+). The majority of PENs (76%, 34/45) were CK7-/CK20-, whereas 16% were CK7+/CK20-, 7% were CK7-/CK20+, and 2% were CK7+/CK20+. The majority of PENs (87%, 39/45) were negative for CDX2, but were often positive for CA19.9 (62%, 28/45).

Conclusions: The diagnosis of PENs on biopsy can present a difficult challenge to the pathologist. Prior studies of CK7/CK20, CDX2 and CA19.9 expression in PENs have evaluated limited cases, and were not performed on TMAs (that simulate biopsy sampling). Our study showed the most common immunophenotype of PENs is CK7-/CK20- (76%), followed by CK7+/CK20- (16%). CDX-2 immunoreactivity was present in 13% of PENs. CA19.9, often regarded as an exocrine marker associated with pancreatic ductal adenocarcinoma, was at least focally positive in the majority (62%) of our PENs. The immunoprofile of PENs with respect to CK7/CK20/CDX2 and CA19.9 overlaps with the immunoprofile of pancreatic adenocarcinoma, and, in cases of non-classic PEN histology, may be a diagnostic pitfall if a limited immunopanel is performed.

540 CpG Island Methylator Phenotype (CIMP) in Microsatellite Stable Colorectal Cancers; Differences between Existing CIMP and Newly Described CIMP

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Background: CpG island methylator phenotype (CIMP) has been recently reported to play a role in colorectal carcinogenesis. Little is known about the causes or results of CIMP independent of microsatellite instability (MSI) in molecular and clinical aspects. Moreover, the consensus of items or criteria determining CIMP has not been yet made. Recently, Weisenberger et al. reported the new CIMP markers. We aimed to investigate the molecular or clinicopathological differences of CIMPs with different standards in sporadic microsatellite stable (MSS) colorectal cancers (CRCs).

Design: Tumor tissues were obtained from 115 patients with sporadic MSS CRC who had undergone curative surgery at Seoul National University Hospital, Seoul. Formalin fixed, paraffin-embedded tissues were used for DNA extraction. CpG island methylation was analyzed using methylation specific PCR described previously. Previously reported CIMP (old CIMP) was determined with 5 CpG island loci; *p16*, h*MLH1*, MINT1, MINT2, and MINT31. Newly described CIMP (new CIMP) was determined using *CACNA1G, IGF2, NEUROG1, RUNX3*, and *SOCS1*. Clinicopathological information was reviewed. Statistical analysis using SPSS software (version 12.0, Chicago, IL) was done.
Results: According to old CIMP's item, 48% of tumors showed methylation at one or more CpG loci. When analyzed with new CIMP's item, 59% of CRCs have methylation at least on one CpG islands. Old CIMP-high tumors (cutoff value=2) and new CIMP-high CRCs (cutoff value=3) were 27.8% (32/115) and 14.4% (16/111), respectively. Both new CIMP-high and old CIMP-high tumors were more frequent in proximal colon cancer (p=0.003 and p=0.02, respectively, Pearson χ^2 test) and old CIMP-high tumors were associated with lymph node (LN) metastasis (p=0.03, Fisher's exact test). New CIMP-high tumors were associated with wild type of *KRAS* codon 12 or codon 13 and *BRAF* codon 600 mutation (p=0.05 and p<0.001, Fisher's exact test), while old CIMP-high tumors were not associated with neither *KRAS* mutation nor *BRAF* mutation. CIMP-high tumors showed worse overall survival compared with CIMP-low tumors of both old and new types (p=0.029 and p=0.38, respectively, Kaplan-Meier log rank test).
Conclusions: Our study showed that new CIMP was associated with *KRAS/BRAF* mutation in MSS tumors, while old CIMP was associated with LN metastasis or poor overall survival.

541 Muscularis Mucosae Duplication in Endoscopic Mucosal Resections for Barrett Esophagus

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Background: Endoscopic mucosal resection (EMR) is increasingly used for management of Barrett esophagus (BE)-related neoplasia. Duplication of the muscularis mucosae (MM) has been described in BE esophagectomy specimens, where it can pose difficulties with accurate staging of carcinoma. The frequency and characteristics of MM duplication in EMRs have not yet been evaluated.
Design: We studied 122 EMR specimens from 100 patients from 1999-2006. Ninety were selected randomly while 22 and 10 were chosen because of a diagnosis of intramucosal (IMAC) and invasive (INV) adenocarcinoma, respectively. The following histologic features were scored: depth of EMR, MM duplication, prolapse changes (extension of smooth muscle into lamina propria), gland entrapment, and diagnosis (original and study/final). Carcinomas reaching the level of submucosa were classified as INV; those confined to lamina propria or MM were classified as IMAC.
Results: Of 122 EMRs, 11 (9%) reached mucosa only, 109 (89%) extended to submucosa, and 2 (2%) extended into muscularis propria. MM duplication was present in 67% (75 of 111 specimens that reached at least submucosa). Prolapse changes were noted in 65 (54%) cases and gland entrapment in 67 (56%). Final pathologic diagnoses were: 9 (7%) no Barrett mucosa (No BE), 4 (3%) BE without dysplasia (DO), 13 (11%) low grade dysplasia (LGD), 51 (42%) high grade dysplasia (HGD), 34 (27%) IMAC, and 12 (10%) INV. EMRs without BE were less likely to show MM duplication (p=0.01) and there was a trend toward less prolapse change (p=0.08) and gland entrapment (p=0.08) as compared to EMRs with BE. However, there were no significant differences with respect to MM duplication, prolapse changes, or gland entrapment between BE with DO, LGD, HGD, IMAC, or INV. Among 34 cases of IMAC, tumor invaded lamina propria in 10 (30%), inner or single MM in 14 (42%), space between duplicated MM in 5 (15%), and outer MM layer in 4 (12%). Lymphatic invasion was seen in 2 cases in which tumor reached the space between MM layers. Two cases of IMAC had originally been classified as INV based on misinterpretation of the muscular anatomy. One case of INV had originally been diagnosed as showing invasion into muscularis propria, when in fact there was invasion through duplicated MM into superficial submucosa.
Conclusions: MM duplication is commonly seen in BE and is also reflected in EMR specimens. Pathologists need to be aware of the peculiar anatomy of BE for accurate staging of carcinomas. Level of IMAC may be a critical feature because of potential access to lymphatic spaces between duplicated MM layers.

542 Skp2 Is an Independent Prognosticator of Gallbladder Carcinoma among p27^{Kip1}-Interacting Cell Cycle Regulators

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Background: Despite improvement in surgical techniques, prognosis of gallbladder carcinoma (GBC) remains poor. It is desirable to identify prognostic biomarkers to aid in the development of targeted therapeutic strategies. Two SCF^{Skp2} ubiquitin ligase-related proteins, Skp2 and Cks1, are involved in post-transcriptional degradation of p27^{Kip1} tumor suppressor, which inhibits the activity of cdk2/cyclin E and cdk2/cyclin A complexes and thus prevents transition to the S-phase. However, the prognostic utility of p27^{Kip1}-interacting cell cycle regulators has not been systematically assessed in GBC.
Design: Immunohistochemistry was performed for p27^{Kip1}, Skp2, Cks1, cyclin E, cyclin A, and Ki-67 in tissue microarrays of 62 GBCs. Only cases with 2 preserved cores were scored. The data were correlated with clinicopathological features and overall survival (OS).
Results: There were 22 men and 40 women with ages ranging from 48 to 89 years (median, 68). Follow-up was obtained in all cases with a median of 29 months. The cumulative OS rate for all 62 cases was 42.9% at 3 years. 27, 29, and 27 cases presented with deep invasion (T3), advanced stage (stage 2, 3, 4), and higher grade (grade 2, 3, 4), respectively. Aberrant labeling indices (LIs) of p27^{Kip1} (<20%), cyclin E (≥5%), cyclin A (≥5%), Cks1 (≥40%), and Skp2 (≥10%) were identified in 29%, 58%, 66%, 21%, and 57% of GBCs, respectively. By log-rank tests, downregulation of p27^{Kip1} (p=0.0319) and high LIs of Skp2 (p=0.0006), Cks1 (p=0.0460), cyclin E (p=0.0070), and Ki-67 (p=0.0037) were predictive of inferior OS. Furthermore, the combined expression status of Skp2 and Ki-67 robustly defined three prognostically different groups (p=0.0001). In multivariate comparison, Skp2 overexpression represented the strongest independent adverse prognosticator (p=0.004, risk ratio [RR]: 5.538), followed by Ki-67 LI ≥50% (p=0.016, RR: 3.254) and AJCC stages II to IV (p=0.013, RR: 3.163).
Conclusions: Aberrations of p27^{Kip1}-interacting cell cycle regulators were common in GBC. Skp2 overexpression is highly representative of biological aggressiveness and independently associated with poor OS. The combined assessment of Skp2 and Ki-67 LIs effectively risk-stratifies GBCs with different prognosis. The findings suggest that Skp2 may be considered as a novel target for therapeutic intervention in aggressive GBC.

543 Tumor Invasion of Altered Lymphatic Channels Is More Prevalent in Small Bowel Versus Rectal Neuroendocrine Tumors: Implications for the Role of Tumor Vascularization in Determining Biologic Behavior

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Background: Despite similar morphologies, small intestinal neuroendocrine tumors (carcinoids) have a higher probability of metastasis than rectal neuroendocrine tumors. While certain parameters such as size and mitotic index have been correlated with prognosis, these parameters alone do not explain the different biologic behavior of these tumors. The monoclonal antibody D2-40 is a specific lymphatic endothelial marker. We used this antibody to compare the extent of tumor lymphatic invasion and total lymphatic density (intratumoral and paratumoral) in a series of neuroendocrine tumors of the small intestine and rectum to determine if these parameters correlate with their known differing biologic outcomes.
Design: A total of 25 cases of neuroendocrine tumors (12 small intestinal, 13 rectal) were examined. Detection of D2-40 expression on paraffin sections utilized a monoclonal antibody and standard immunohistochemical techniques. The presence or absence of tumor in partial or complete D2-40 expressing lymphatic spaces was determined. The intratumoral and paratumoral lymphatic density was evaluated in ten high-power fields (HPF; 40X).
Results:

Table 1

Site (# of cases)	# of cases with tumor in lymphatic spaces		# of lymphatic spaces/ 10 HPF Mean±SD	
	Partial D2-40 staining	Complete D2-40 staining	Intratumor	Paratumor
Small Intestine (12)	5 (42%)	5 (42%)	14±14	25±14
Rectum (13)	1 (8%)	4 (31%)	10±9	24±14

There was no significant difference in intra- and paratumoral vessel density as well as presence of tumor in complete lymphatic spaces between these two groups. However, tumor invasion in lymphatic spaces partially expressing D2-40 was observed in 5 of 12 (42%) small intestinal carcinoids, in contrast to only 1 of 13 (8%) rectal carcinoid tumors (Chi-Square test, p<0.05).
Conclusions: Despite similar lymphatic densities and tumor invasion in complete lymphatic spaces in these two biologically disparate tumors, the finding of increased tumor invasion in incomplete/altered lymphatic channels in the more aggressive small bowel neuroendocrine tumors compared to the indolent rectal tumors raises the possibility that vascularization is indeed an important factor in predicting the metastatic ability of these tumors. The mechanisms of the altered expression of D2-40 in these spaces are not known; however, the possibility of variable tumor-endothelial interactions by these neoplastic cells will be explored.

544 Evaluation of AMACR (P504S) Expression Patterns in Reactive, Dysplastic and Malignant Gastric Tissues

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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, and is overexpressed in various normal tissues and malignant neoplasms, such as prostate and colon cancer. Recent studies have demonstrated increased expression of P504S in dysplasia and adenocarcinoma in patients with Barrett's esophagus and inflammatory bowel disease (AJSP 30:871). Due to interobserver variability and overlapping morphologic features, distinguishing gastric dysplasia from reactive changes can be difficult and an immunohistochemical marker that would aid in this differential would be useful. The aim of this study is to assess the staining patterns of P504S in reactive gastropathy, gastric dysplasia and adenocarcinoma.
Design: Tissues and records were obtained from the archives of the Medical College of Georgia and the Augusta VAMC. Study cases included 10 biopsies of reactive gastropathy, 12 biopsies of gastric dysplasia (from 8 patients), and 10 resections of gastric adenocarcinoma. Replicate 5μ sections were evaluated with hematoxylin and eosin staining to confirm the presence of lesional tissue. P504S immunohistochemical staining was performed on all cases. Positive (prostatic adenocarcinoma) and negative controls reacted appropriately. The degree of cytoplasmic immunostaining was scored as diffusely positive (3+), focal positive (2+), rare positive (1+), or negative in a blinded fashion by all authors.
Results: All cases (100%) of reactive gastropathy were negative for P504S staining. 6 of 12 (50%) cases of dysplasia stained positive for P504S (2=3+; 4=2+) . Two patients had three separate biopsies each prior to resection; all biopsies in these cases had correlative staining (one positive in all biopsies and one negative). 6 of 10 adenocarcinomas demonstrated positive staining (1=3+; 3=2+; 2=1+). 3 biopsy cases of dysplasia had surgical resections, and correlative staining was identified in 2 of 3 patients.
Conclusions: Increased P504S expression was observed in 50% of gastric dysplasias and 60% of gastric adenocarcinomas whereas reactive gastropathies were consistently negative (100%). Our findings indicate that P504S staining may be useful in differentiating dysplasia and adenocarcinoma from reactive change. Though negative P504S staining does not exclude dysplasia or adenocarcinoma, positive staining should alert the pathologist to the possibility of dysplastic or malignant change.

545 Lymphatic Microvessel Density as Prognostic Marker in Esophageal Adenocarcinoma

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Background: Metastasis to regional lymph nodes serves as a major prognostic indicator for disease progression and as a guide for therapeutic strategies. Recent studies showed that D2-40 is a specific lymphatic marker. There is limited data evaluating the significance of lymphatic microvessel density (LMD) as a prognostic marker in patients with esophageal adenocarcinoma. In this study, we investigated tumor lymphangiogenesis as a predictive marker for the risk of lymph node (LN) metastasis and its relation to other prognostic parameters and survival in esophageal adenocarcinoma patients.

Design: Surgical specimens from 75 patients with esophageal adenocarcinoma treated with esophagectomy were immunostained for D2-40, CD31, and VEGF. We also included 10 cases of Barrett's esophagus with high-grade dysplasia (BE-HGD). 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. Intensity of staining for VEGF was scored on a three-tiered scale. Results were correlated with other prognostic parameters including survival.

Results: D2-40 LMD demonstrated significant correlation with CD31 MV counts (20+/-9 vs 11+/-6/0.17 mm2 field, P<0.05). CD31 MV counts showed significant correlation with patient survival (log rank P< 0.01). However, there was no correlation between CD31 MV and presence of angiolymphatic invasion, lymph node metastases and disease stage. D2-40 LMD demonstrated a significant correlation with lymph node metastases (r=0.31, P< 0.01), presence of angiolymphatic invasion (r=0.41, P<0.01), but not tumor stage. On statistical analysis, D2-40 LMD demonstrated significant correlation with shorter disease free, but not with overall survival. D2-40 detected angiolymphatic invasion in 27/75 cases, more than with CD31 (23/75) and H&E (18/75). There is was no significant difference between BE-HGD and adenocarcinoma MV density. VEGF was expressed in 48/75 (64%) cases of adenocarcinoma and was significantly correlated with LN metastases, and overall survival.

Conclusions: Our study showed that both angiogenesis and lymphangiogenesis play an important role in the progression of esophageal adenocarcinoma. D2-40 LMD showed prognostic significance with positive correlation with angiolymphatic invasion, metastases to lymph nodes and shorter disease-free time. In addition, D2-40 increases the frequency of detection of lymphatic invasion relative to conventional H&E staining and the commonly used pan-endothelial marker, CD31.

546 Reduced Immunoexpression of O^6-Methylguanine-DNA Methyltransferase (MGMT) in Inflammatory Bowel Disease (IBD)-Associated and Sporadic Serrated Polyps

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Background: Inactivity of DNA repair genes has been implicated in the hyperplastic-adenoma-carcinoma carcinogenic pathway in the colon and considered as an important mechanism of tumorigenesis in colon cancer with microsatellite instability (MSI). Hypermethylation and reduced expression of MGMT, a DNA repair gene involved in the removal of alkyl groups from the O^6 position of guanine, has been identified in MSI colon cancer as well as in sessile serrated polyps. Expression of MGMT, however, has not been evaluated in serrated lesions arising in a background of IBD. Additionally, the clinical significance of IBD-associated serrated lesions has not been evaluated. As a result, we undertook these studies to determine to what extent IBD-associated serrated lesions resemble their sporadic counterparts.

Design: Fourteen serrated polyps in patients with established IBD (9 ulcerative colitis-associated, 3 right and 6 left colon; 5 Crohn's disease-associated, 2 right and 3 left colon) and 10 sessile serrated polyps in non-IBD patients (4 right and 6 left colon) were evaluated. Immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue using a monoclonal antibody to MGMT (Chemicon International, 1:75). The result was interpreted as no loss, superficial (top half of the crypt) loss and complete loss of MGMT immunoexpression in the serrated epithelium.

Results: Nuclear staining for MGMT was consistently evident in the non-serrated epithelium and some inflammatory cells in the lamina propria, which were used as an internal control. Loss of MGMT was found in 8 (overall 57%, 8/14) IBD-associated serrated polyps (2 complete, 6 superficial loss), compared to 8 (overall 80%, 8/10) of non-IBD associated sessile serrated polyps (2 complete and 6 superficial loss). Among the 8 IBD-associated serrated polyps with loss of MGMT expression, 5 were UC-associated, including 2 polyps of the right colon with complete loss and 3 polyps of the left colon with superficial loss; The remaining 3 polyps with superficial loss of MGMT expression were Crohn's disease-associated, one in right colon and 2 in the left colon.

Conclusions: MGMT expression appears to be downregulated not only in sporadic sessile serrated polyps in non-IBD patients but also in IBD-associated serrated polyps in this pilot study. Further studies are in progress to examine the association between DNA methylation of MGMT and clinicopathological features of IBD-associated serrated polyps.

547 Synovial Sarcoma of the Stomach – A Clinicopathologic, Immunohistochemical and Molecular Genetic Study of 6 Cases with a Long-Term Follow-Up

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Background: Only few synovial sarcomas arising in the gastrointestinal tract have been reported, mostly from the esophagus and one in the stomach.

Design: We reviewed clinical, histopathologic, and immunohistochemical features of 6 gastric synovial sarcomas accessioned in the Armed Forces Institute of Pathology. During the same period over 2000 gastric GISTs were seen.

Results: The tumors occurred in 3 males and 3 females with median age of 54 years (range, 29-68 years). Two tumors were large masses of 8 and 15 cm, and 4 were 2-3 cm ulcerated plaque like lesions. Three of the 6 cases were initially interpreted as leiomyosarcoma, 2 as spindle cell neoplasm of uncertain malignant potential and only one was diagnosed as synovial sarcoma. Histologically, all but one tumor were monophasic. Microscopic calcifications were present in one tumor. One biphasic and 4 monophasic tumors tested were focally positive for EMA and cytokeratin. Vimentin was positive in all 4 tested tumors. SYT-SSX fusion transcripts were demonstrated in 3 cases studied by PCR-assay. All tumors were totally excised; 3 patients had partial gastrectomy and 3 underwent segmental resection. None of the patients had received postoperative radiation. Follow-up showed that 3 patients were alive and well 21-224 months after surgery (median 22 months). Two patients had died of the tumor 25 and 29 month after surgery, of whom one had local omental recurrence 13month after diagnosis and the other had postoperative chemotherapy. One patient was lost for follow-up.

Conclusions: Synovial sarcoma can rarely occur as a gastric primary. It has a variable prognosis depending on tumor size and should be considered in the differential diagnosis of gastric spindle cell neoplasms.

548 Assessment of Mucin Pools in Rectal Carcinoma Post Neoadjuvant Chemoradiotherapy

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Background: The presence of mucin pools (MPs) in the resection specimens of post neoadjuvant chemoradiotherapy (CRT) rectal carcinoma is a well recognized phenomenon. The current recommendation by the College of American Pathologists (CAP) is to exclude acellular MPs in the classification by ypTNM. However, conflicting reports exist with regard to the clinical relevance of such MPs.

Design: The pattern and significance of MPs were analyzed in 108 prospectively collected resection specimens of treated rectal carcinoma. All 108 patients had uT3-4 and/or uN1 rectal cancer, and were treated with pre-operative long course CRT. All specimens were handled uniformly: residual tumor region was entirely submitted for microscopic examination using wholemount sections to encompass all layers of the rectal wall, and lymph nodes (LNs) thoroughly examined. A prospective clinical database was maintained.

Results: There were 39 female and 69 male patients; median age 58.5 years. MPs were identified at the primary tumor site in 33 of the 108 cases (31%), 16 (15%) were acellular. The MPs were focal (<10% of the entire lesion) in 14, and extensive (>50%) in 8. MPs were also noted in the LN in 6 cases (6%), 3 were acellular. Five cases had MPs in both the primary site and the LNs. When acellular MP was considered as "no residual tumor", the complete pathological response (CPR) rate was 22% (24/108); the pathologic stage of the residual tumor (ypT) was 0 or 1 for 27 cases and 2-4 for 81; and the ypN was 0 for 83 and 1 or 2 for 25. When acellular MP was considered as "residual tumor", the CPR dropped to 16%; the ypT was upstaged in 10 tumors (6 from 0 to 2 or 3, 4 from 2 to 3); and ypN was upstaged in 2 (1 from 0 to 1, and 1 from 1 to 2). With a median follow up of 31 months, 23 patients recurred. The estimated 3-year recurrence free survival (RFS) was 73%. Neither the presence of MPs nor their extent or cellularity correlated with RFS. Further, none of the 12 patients whose ypT or ypN was upstaged by acellular MPs had recurrent disease. The 3-yr RFS in these patients was 100%, in contrast with 65% observed in patients who were staged ypT2 or T3, and 60% who were staged ypN1 or N2, by viable tumor cells.

Conclusions: With a well controlled group of study subjects, our results suggest that MPs in treated rectal cancer do not have a significant impact on patient outcome. Our data support the CAP recommendation that only viable tumor cells, not acellular MPs, are to be interpreted as residual disease and classified by ypTNM.

549 p16 Staining Improves Interobserver Agreement in Diagnosing Anal Intraepithelial Neoplasia

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Background: The histological evaulation of anal intraepithelial neoplasia (AIN) is reported to be quite subjective and is prone to both inter- and intraobserver variation. Recent studies demonstrate that p16 expression correlates with the degree of AIN. The objectives of this study are (1) to determine the extent of interobserver agreement in evaluating AIN based on routine H&E sections and (2) to determine if p16 staining improves interobserver agreement.

Design: Seventy-seven anal biopsy and resection specimens were retrieved. Additional sections were obtained for immunostaining with monoclonal antibody against p16 (clone: 16P04; dilution 1:100; Cell Marque, Hot Springs, AR). Blind to the original diagnoses, 5 pathologists independently assessed both H&E sections and p16 stained slides and assigned them into four diagnostic categories: normal/reactive, AIN I/HPV, AIN II, and AIN III.The agreement in the diagnosis between observers was calculated by kappa statistics.

Results: All pathologists were board-certified in anatomic pathology and had experience ranging from 2 years to 25 years (mean=13.6 years). Based on H&E preparation, 18 (27%) cases had a unanimous agreement and included 7 benign/reactive lesions, 3 AIN I/HPV, 1 AIN II, and 7 AIN III. The pair-wise kappa values ranged from 0.32 to 0.50 with a mean of 0.38. Based on p16 staining, 38 (49%) cases had a unanimous agreement and included 29 benign/reactive lesions, 3 AIN I/HPV, 2 AIN II, and 4 AIN III. The pair-wise kappa values ranged from 0.50 to 0.74 with a mean of 0.57. The difference in the kappa values between H&E preparation and p16 staining was statistically significant (t-test, p=0.001). When AIN II and AIN III were combined into a single category, the mean kappa values were 0.42 and 0.67 based on H&E preparation and p16 staining, respectively; however, the increases in kappa values were not statistically significant (t-test).

Conclusions: The interobserver agreement in diagnosing AIN was poor when based solely on H&E preparation. p16 immunostaining improved interobserver reproducibility in the histopatholgoic interpretation of anal specimens, particularly in the diagnosis of normal/reactive cases. Therefore, p16 immunostaining may reduce false positive and false negative biopsy interpretation thereby improving the diagnostic accuracy of AIN in biopsy specimens.

550 Pathology of Barrett Esophagus after Photodynamic Therapy

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Background: Photodynamic therapy (PDT) is increasingly used for the treatment of Barrett Esophagus (BE) and its related superficial neoplasia. It, in turn, has increased the opportunity to review biopsies obtained after PDT. The risk of squamous re-epithelization overriding residual BE and/or neoplasia post PDT has been reported. However, little is known about other pathologic features, including potential diagnostic pitfalls, in post-PDT biopsies.

Design: 23 patients with BE related neoplasia (mean age: 71 yr, M:F=18:5, mean BE length: 6.5 cm) formed our study group. A pre-PDT biopsy containing both non-dysplastic and dysplastic mucosa was selected in each case and those obtained from the same anatomic level 3 months (3M), 6-9 months (6M) and 12-18 months (12M) after one course of PDT were also examined. Several features were semiquantitatively evaluated (grade 0-2) in the epithelium (presence of goblet cells, apoptosis, atypia), stroma (capillary proliferation, edema, fibromuscular expansion), inflammatory components and architecture (atrophy). A fraction of tissue surfaced by squamous epithelium and squamous atypia were also recorded.

Results: The surface lined by squamous epithelium was significantly increased post PDT and no squamous atypia was noted. In residual non-dysplastic BE, goblet cells were significantly decreased at 3M. Although reactive atypia was more common post PDT, it was usually mild. Capillary proliferation and eosinophilia were significant at 3M and 6M but subsided thereafter. Fibromuscular proliferation was most prominent at 6M and correlated to glandular atrophy. Similar tendencies were observed in dysplastic mucosa; however, statistical difference could not be determined because of, as expected, significantly decreased number of biopsies containing dysplastic tissue post PDT.

Conclusions: After a single course of PDT, a broad area of BE is replaced by squamous epithelium without atypia. In the residual BE reparative changes were prominent at 3M and 6M but subsided at 12M. Reactive epithelial atypia, the possible mimic of residual neoplasia, was usually mild and not diagnostically challenging.

Representative pathologic changes in non-neoplastic BE

	SQ (%)	Goblet cells*	Atypia*	Capillaries*	Fibromuscular expansion*	Eosinophils*	Atrophy*
P	4.78**	1.13	0.26	1.05	0.74	0.50	0.21
3M	31.70**	0.71**	0.88**	1.53**	1.09	1.15**	0.71**
6M	49.40**	0.71	0.79	1.50**	1.32**	1.14**	0.86**
12M	64.32**	0.82	0.64	1.36	1.23**	1.09**	0.73**

* mean grade, ** < 0.05 compared to pre PDT

551 Duplicated Muscularis Mucosa in Barrett Esophagus – Vascular Characteristics of *Newly Formed* Submucosa

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Background: The mucosa of metaplastic Barrett esophagus (BE) is frequently associated with duplicated muscularis mucosa (MM). Although it has been suggested that the outer MM replicates the *original* MM, BE adenocarcinomas invading the duplicated MM can fare poorly despite their suggested intramucosal location. Interestingly, little is known about the physiologic characteristics of the *newly formed* submucosa (NSM) - a space between the inner MM and outer MM. The aim of this study was to compare the density of angiolymphatic vessels in the NSM with that in the submucosa (SM) as a way to evaluate the potential risk for metastasis in neoplasms invading the NSM compared to those invading the SM.

Design: Our study group consisted of 16 esophagectomy specimens for BE related superficial neoplasia (M:F=14:2, mean age: 63 years, mean length of BE: 4.3 cm). A section of the squamo-glandular junction containing non-neoplastic Barrett mucosa with duplicated MM was selected in each case. Immunohistochemistry for D2-40 and double immunohistochemistry for CD31 and smooth muscle actin were carried out on each section. The number of D2-40 positive lymphatic vessels and CD31 and smooth muscle actin double positive muscular vessels are counted in three 200x microscopic fields in the lamina propria of squamous mucosa (SqLP), NSM and SM by 2 observers independently. An average number of the angiolymphatic vessels in a 200x field was calculated for SqLP, NSM and SM on each section and compared.

Results: SqLP and NSM contained similar number of lymphatic vessels. Their number was distinctly higher than in the SM. Significantly, a larger numbers of muscular vessels were also seen in SqLP and NSM than in SM. In addition, larger caliber and thick muscular wall vessels were usually observed in SM, but rarely noted in either SqLP or NSM.

Conclusions: The *newly formed* SM of BE, due to a duplication of MM, preserves the vascular profile of SqLP. The reported aggressiveness of tumors invading the duplicated MM may be attributed to their own biologic characters and features such as a size/radial dimension, but not to an increase in angiolymphatic structures.

A number of vessels in a 200x field

	Lymphatic vessels	Muscular vessels
SqLP	9.21 ± 4.89^{a}	32.70 ± 7.15^{c}
NSM	9.77 ± 4.88^{b}	34.44 ± 9.39^{d}
SM	2.99 ± 1.80^{ab}	22.96 ± 8.65^{cd}

a, b, c: $p < 0.001$, d: $p < 0.01$

552 4EBP1 Overexpression, a Cornerstone in Cell Signalling, Associates with High Risk GIST and Support the Distinction between Low and High Risk GISTS

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Background: The expression of Kit and its different mutations are compulsory diagnostic traces for Gastrointestinal Stromal Tumors and represent a therapeutic target for imatinib (Glivec®). Nevertheless, only high risk GISTS are treated and importantly there are a subgroup of "intermediate" risk of GISTS that can progress to high grade or show a malignant clinical behavior. In this study, we have analyzed the signaling pathways in these tumors in order to detect factors associated with different biological behavior. Because in 4EBP1 converge several upstream pathways, we hypothetized that p4EBP1 overexpression could reflect the real oncogenic activation of these pathaways and predict risk of progression in GISTS.

Design: Tissue-microarrays blocks were constructed with *121* formalin fixed cases of GIST. All cases were classified as high-intermediate risk or low-very low risk using Fletcher prognostic risk score. For each case, three selected cylinders of 1 mm from different and representative tumor areas were included (111 cores). Also, normal tissue from the same specimen was used as control. Immunohistochemical assay with 4EBP1 (Cell signaling) with the Envision method was performed. The percentage of positive cells, intensity and localization of the staining were compared. Intensity and percentage of immunoexpression was scored on a scale from 0 to 300 in each case. Other markers such as pAKt, pAKt and ki67 were also analyzed. Mutational study of c-kit was performed in 85 cases. Data was analyzed using SPSS 10.0 sofware.

Results: With the results obtained, only we obtained significant clinico-pathological associations with p4EBP1. The 4EBP1 expression was significatively higher in the group of high /intermediate risk than those of low/very low risk tumors (P=0.003). Thus, in most of the cases of high/intermediated risk group, the expression was located in both nuclei and cytoplasm, which differ to the low/very low risk tumors where the expression was mainly nuclear (P=0,044).

Conclusions: p4EBP1, a pivotal factor in cell signaling trasduction, is associated with the more aggressive group of GIST. p4EBP1 could be a predictive biomarker for aggressive biological behavior in GIST and distinguish molecularly the low and high risk GISTS helping to include the "intermediate" group in either high or low risk GIST depending on the level of overexpression of p4EBP1.

553 Gastrointestinal CMV Disease: Plasma Viral Load, In-Situ Hybridization, Immunohistochemistry, and Clinicopathologic Findings

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Background: Gastrointestinal cytomegalovirus (CMV) disease is a significant cause of morbidity and mortality in immunocompromised patients. Few studies have correlated its clinical and pathological findings. We correlated the prevalence and magnitude of CMV viremia with tissue CMV by in-situ hybridization (ISH) and immunohistochemistry (IHC) among biopsy-proven gastrointestinal CMV disease patients.

Design: 38 biopsies (30 patients) from various sites in the GI tract with CMV cytopathic effect were studied. CMV plasma viral loads were assayed by PCR within10 days of tissue sampling. CMV ISH and IHC were performed, using the Bond-max autostainer (Vision BioSystems) with prediluted CMV probe, and NCL-CMV pp65 monoclonal antibody (1:500 dilution) (Novocastra), respectively. CMV plasma viral load was correlated with: 1- number of infected cells per 10 HPF in biopsy specimens (ISH, IHC); 2- evidence of disease in multiple sites within the GI tract; 3- presence of systemic/extra GI symptoms; 4-endoscopic findings; 5- underlying medical condition predisposing to CMV infection.

Results: 86.6% of patients had detectable viremia, with a mean viral load of 21,943 copies/ml (range 350 to >50,000). The average number of CMV-infected cells was 20 per 10HPF by ISH and 39.5 per 10HPF by IHC, with no correlation with plasma viremia. We found higher mean viral loads in patients with positive biopsies in multiple GI sites as compared to patients with CMV lesions in a single site (26,985 and 19,449 copies/ml respectively), in patients with extra-GI symptoms compared to symptoms limited to the GI tract (29,600 and 15,895 copies/ml, respectively), and in patients with ulceration/erosion seen on upper endoscopy compared to any other finding (28,568 and 17,400 copies/ml). Solid organ transplant and HIV patients tended to have higher viral loads (31,730 and 27,550 copies/ml, respectively) compared to bone marrow transplant and immunocompetent patients (10,804 and 2,850 copies/ml, respectively). These differences did not achieve statistical significance, likely due to our small number of patients.

Conclusions: Most cases of GI tract CMV disease diagnosed by biopsy are associated with positive viremia. We found no correlation between the number of infected cells in tissue and viral load. Our results suggest that gastrointestinal CMV is usually part of a systemic infection, occurring most commonly in immunocompromised individuals, in whom higher viral loads tend to be associated with more aggressive infection involving multiple organs.

554 Diagnostic Utility of Alpha-Methilacyl-Coa Racemase (AMACR) in Digestive System Neoplasms

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Background: AMACR is a mytocondrial and peroxisomal enzyme involved in the β-oxidation of dietary branched fatty acids. It was initially described as a diagnostic marker of prostatic carcinoma, although recent reports indicate its expression in other neoplasms, especially in colorectal carcinoma. The purpose of this study was to perform a wide screening of AMACR expression in the digestive system and to determine its frequency in selected types of neoplasms.

Design: Tissue microarrays (TMA) were elaborated from the surgical pathology archives and included normal tissues and the most frequent neoplasms of the digestive system. Specific TMA representing normal, preneoplastic and neoplastic tissues from pancreas

(26 infiltrating adenocarcinomas), liver (42 hepatocarcinomas) and colon (30 infiltrating adenocarcinomas) were also constructed. Immunohistochemical studies were carried out using antiAMACR (Policlonal, 1:100, Dako) and the Envision System (Dako). The staining was classified as negative, weak or strong, according to intensity and percentage of positive cells.

Results: Weak AMACR expression was observed in hepatocytes and scattered cells of colonic crypts. Seventy six percent of hepatocarcinomas were strongly positive and showed a significant stronger AMACR expression when compared to normal and cirrhotic liver ($p=0.015$) and to high grade displastic nodules ($p=0.023$). We found no differences in AMACR expression when considering TNM of hepatocarcinomas, and between normal and cirrhotic liver. Two thirds of colon cancers were also strongly positive. We also observed a weak positivity in twenty percent of pancreatic tumors. No statistically significant differences were observed when considering TNM parameters in this later types of tumors. Preneoplastic lesions (high grade displastic nodules, colonic adenomas) showed a weaker AMACR expression than liver and colonic carcinomas. Samples of PANIN were negative. The rest of gastrointestinal samples of normal and neoplastic tissues were negative.

Conclusions: Our wide screening of AMACR expression showed a frequent and intense positivity in several neoplasms of the digestive system, including liver and colon. This results suggest that AMACR could have a role in the development of tumors of the gastrointestinal tract, and it may be an useful marker in the differential diagnosis of metastatic neoplasms of unknown origin, especially in women, whereas such positivity must not be considered pathognomonic of prostatic tumors in men.

555 Clinical Significance of Colonic Intraepithelial Lymphocytosis in the Pediatric Population

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Background: Colonic intraepithelial lymphocytosis (CIL) has been well described in adult populations and is present in conditions such as microscopic colitis, celiac disease (CD), and NSAID use. However, little is known about the significance of CIL and its disease associations in the pediatric population. This study was undertaken to examine these disease associations in a cohort of children with CIL.

Design: 27 cases (25 pts) were identified in which CIL was a prominent feature between 1994 and 2004. CIL was defined as 20 or more intraepithelial lymphocytes (IELs)/100 enterocytes in the surface epithelium. Each biopsy was examined for the following: crypt architecture distortion, cryptitis, crypt abscesses, basal lymphoid aggregates (BLA), increased subepithelial collagen, surface ulceration, granulomas, increased lamina propria chronic inflammation (LPCI), and metaplastic epithelium. Indications for biopsy, clinical follow-up, final pathologic diagnosis, associated comorbidities, autoimmune serologies, and treatment regimens were also tabulated.

Results: 121 colonic biopsies were examined in patients ranging from 1-17 years (mean 10.2 years; 48% male) in which a mean of 29 (±22) IELs per 100 enterocytes were seen. Common indications for endoscopy included diarrhea (16 pts) and abdominal pain (9 pts). 14 patients had CIL as the only histologic finding. Focal cryptitis, Paneth cell metaplasia, LPCI, BLA, architectural distortion, crypt abscesses and ulceration were seen in 5, 5, 4, 3, 3, 2 and 2 patients respectively. Positive anti-TTG and/or anti-endomysial antibodies were noted in 5 of 12 patients. The mean follow-up period was 1.2 years (0 to 4 years). Inflammatory bowel disease was diagnosed in 7 of 25 patients and was seen only in cases in which CIL was present with architectural abnormalities or inflammatory changes. IgA deficiency, CD, lymphocytic colitis, multisystem autoimmune disease, allergic colitis, autoimmune enteropathy were found in 5, 5, 4, 2, 2 and 1 cases, respectively.

Conclusions: Pediatric CIL in the absence of other significant histologic findings is associated with CD, lymphocytic colitis, autoimmune enteropathy, multisystem autoimmune disease and immunodeficiency states, such as IgA deficiency.

556 High JC Virus Load in Gastric Cancer and Adjacent Non-Cancerous Mucosa

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Background: The JC virus (JCV) infects a large proportion of the population worldwide and about 90% of adults are seropositive. Recent reports described the possibility of its oncogenetic role in several malignancies.

Design: The aim of this study is to assess the oncogenetic significance of the JCV for gastric cancer. Twenty-two sample pairs of fresh tumor and adjacent non-cancerous tissue (ANCT), as well as 10 normal gastric mucosa specimens were investigated on the basis of nested PCR followed by Southern bolt, DNA direct sequencing, real-time PCR, in situ PCR and immunohistochemistry.

Results: The *T-antigen* sequence was detected in 86.4% of both gastric cancers and ANCTs, and in 100% of the normal mucosa samples, as for *VP*, 54.1%, 68.1% and 70%, respectively. As to *agno-protein*, generally a low incidence was noted. The JCV DNA load was about 10 folds higher in both gastric cancers and paired ANCTs (4784±759 copies/μg DNA and 5394 ± 1466 copies/μg DNA, respectively), than in normal gastric tissue (542.4±476.0 copies/μg DNA, $p<0.0001$). In situ PCR revealed sporadic JCV genome positive cancer cells and foveolar epithelial cells. T-antigen protein expression by immunohistochemistry was detected only in one case (1/22; 4.5%) probably because a half life of T-antigen might be short.

Conclusions: It is concluded that the gastric epithelium in most Japanese is infected with JCV at a low rate but levels of infection are increased markedly in both cancer cells and ANCT, indicating a possibility that multiplication of JCV copies might be a risk factor and a background for gastric carcinogenesis.

557 Patterns of Immunohistochemical Expression of Folate Receptor alpha (FR*α*) in Colorectal Epithelium and Its Neoplasms

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Background: FRα is a membrane-anchored protein that binds and transports folic acid, a vitamin essential for the biosynthesis of DNA, into cells. Because of its high affinity for the vitamin ($Kd=10^{-10}$M), FRα is often upregulated in rapidly dividing cells, especially cancer cells. Such a property has been exploited for the selective delivery of folate-linked drugs to FRα-expressing cancer cells. However, although three folate-linked drugs are currently in clinical trials, most studies on FRα expression have been performed by RT-PCR which is inherently nonquantitative, or by cyto-fluorimetric assays that require frozen tissue. As such, the number of studies has been limited, and the pattern and significance of FRα expression in many tumor types remain largely to be defined.

Design: Using a monoclonal antibody (mAb343) that was shown to stain FRα in paraffin-embedded tissue and standard tissue microarrays, we studied immunohistochemical expression pattern of FRα in a series of colorectal carcinomas (CRCs), their precursors and normal mucosa, and correlated staining pattern with tumor pathological features and clinical outcome.

Results: FRα staining results are outlined in Table 1. FRα expression in primary CRC did not correlate with any of the pathological features analyzed (mucinous type, histological grade, pathological T or N stage, or lymphovascular invasion). However, patients with 1+ or 2+ staining had a worse recurrence free survival than those that exhibited no staining ($p<0.03$).

FRα staining results.

	Normal mucosa	Adenoma	Primary carcinoma	Metastasis		
				Lung	Liver	Total
FRα = 0*	141 (93%)	39 (93%)	119 (67%)	9 (35%)	20 (77%)	29 (56%)
FRα = 1+*	8 (5%)	2 (5%)	42 (24%)	16 (62%)	4 (15%)	20 (38%)
FRα = 2+*	3 (2%)	1 (2%)	16 (9%)	1 (3%)	2 (7%)	3 (6%)
Total # of cases	152	42	177	26	26	52

* 0 = no staining in any tissue core; 1+ = at least 1 core showing at least weak staining but not qualifying for 2+; 2+ = at least 2 cores showing moderate staining or 1 core showing strong staining.

Conclusions: Our study demonstrates that FRα is upregulated in a subset of colorectal carcinomas while its expression is limited in benign tissues, providing a foundation for further investigation of the use of folic acid conjugates for imaging and therapy of colorectal tumors. Our data also indicate that FRα expression in colorectal tumors may bear prognostic significance, warranting future studies with additional cases.

558 Serrated Polyp Neoplasia Pathway Histological Categories in the National Polyp Study (NPS)

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Background: An alternative pathway to colorectal carcinoma originating in a serrated polyp is now recognized. The aim of this study is to assess the relative distribution of the histological categories of the serrated pathway and their potential contribution to advanced adenomas and carcinomas in the National Polyp Study (NPS) cohort. The NPS is a multi-center randomized clinical trial, completed in 1991, that has been influential in establishing appropriate surveillance guidelines for patients discovered to have one or more colorectal adenomas on colonoscopy.

Design: H&E slides of all colorectal tissues removed, including those at the initial and surveillance colonoscopies of the randomized patients, as well as those with hyperplastic polyps only or colorectal cancer at the initial exam, who were not randomized, were reviewed. Hyperplastic polyps (HP) were re-classified as Goblet Cell (GCSP) or Microvesicular (MVSP) Serrated Polyps or Serrated Polyp with Abnormal Proliferation (SPAP) – syn: Sessile Serrated Adenoma (SSA) based on the criteria of Torlakovic et al. WHO criteria for serrated or mixed polyps (SA) were applied to all adenomas and carcinomas.

Results: The referral (non-randomized) cohort included 88 carcinomas, 53% (n=47) with residual adenoma; among these 6.4% (n=3) had residual serrated adenomas (SCa). The table summarizes HP categories as % of all HPs and SAs as % of all adenomas removed in: a)the referral group with HPs only; b)the randomized adenoma bearing group at baseline and c) the randomized group on follow-up surveillance.

Serrated Polyp Category Frequency by Study Group

Study Group	GCSP/HP	MVSP/HP	SPAP[SSA]/HP	SA/Adenomas
a) HPs only	10.1% (n=44)	83% (n=360)	6.9% (n=30)	--
b) Randomized	14.1% (n=92)	80.5% (n=526)	5.3% (n=35)	0.6% (n=18)
c) Follow-up	16.3% (n=25)	79.1% (n=121)	4.6% (n=7)	0%
		Chi2=6.5; p=0.17		Chi2=4.9; p=0.02

Conclusions: Advanced histological categories of the serrated polyp neoplasia pathway (SPAP/SA/SCa) were represented in the referral cohort and the randomized patient group but only SPAPs (syn:SSAs) and no SAs were found among polyps discovered on follow-up surveillance.

559 Pathologic Features of Carcinomas in Patients with MYH Associated Polyposis

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Background: MYH is a DNA glycosylase involved in the base excision repair process. It is responsible for the removal of adenines mismatched with 8-oxo-guanine, a product of oxidative damage. Biallelic mutations in the MYH gene are associated with the development of multiple colonic adenomas and colorectal carcinoma. A slightly increased risk of colorectal carcinoma has been noted in monoallelic mutation carriers. We examined carcinomas from patients with biallelic and monoallelic mutations and compared them to a control group.

Design: Colorectal carcinomas and polyps from 50 patients(28 females, 22 males) were retrieved from the files of the OFCCR and the Familial Gastrointestinal Cancer Registry. The study group included 56 carcinomas(14 biallelics (including homozygotes and compound heterozygotes), 25 monoallelics and 17 nonpathogenic MYH polymorphisms). Clinicopathologic features were recorded.
Results: The average age at diagnosis was 51(biallelics), 58(monoallelics), and 61 years(controls). Serrated carcinoma was the predominant type in 13.6%(3/25) of the monoallelic group but in none of the biallelic/polymorphism groups. A minor serrated component was a more frequent finding in the biallelics[28% (4/14)] and monoallelics[28%(7/25)] than in the polymorphism group[12% (2/17)], (not significant,p>0.1). The predominant histologic pattern seen most frequently in all groups was tubular[biallelic=57%(8/14); monoallelic=44%(11/25); polymorphism=24%(4/17)]. Common secondary patterns included papillary and cribriform. No significant difference between the groups was noted for tumour site, grade, nature of invasive margin, a poorly differentiated component at the invasive margin, lymphovascular invasion, Crohn's like reaction and tumour infiltrating lymphocytes. The histologic types of the polyps seen in each group were similar (tubulovillous adenomas, hyperplastic polyps and serrated adenomas).
Conclusions: The carcinomas from patients with biallelic and monoallelic mutations in MYH did not differ in pathologic appearance from those in the control group. A minor serrated component was more frequently observed but the difference was not significant. One limitation of this study is the small number of carcinomas from patients with biallelic mutations. Further studies are therefore needed to demonstrate if there are pathologic features predictive of biallelic MYH mutations.

560 Autoimmune Enteropathy-Like Panenteritis Is Associated with the Novel Cancer Therapy Adjuvant a-CTLA-4 mAb

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Background: Monoclonal antibodies (mAbs) against cytotoxic T lymphocyte antigen-4 (CTLA-4) molecule are used in clinical trials as an adjuvant to tumor immunization. The gastrointestinal (GI) side effects induced by this type of biologic therapy have not been well described. The goals of the study were to characterize the GI pathology associated with α-CTLA-4 mAb therapy and test the hypothesis that a depletion of T_{reg} may contribute to the pathology.
Design: We evaluated 5 patients who developed GI toxicity during a clinical trial of α-CTLA-4 mAb for treatment of ovarian carcinoma or malignant melanoma. Colonic biopsies were obtained in all, while gastric and small intestine biopsies were obtained in 1 and 2 patients respectively. Immunophenotyping using mAbs was performed on colonic biopsies. Total $CD3^+$ and $FoxP3^+$ T_{reg} cells were quantified in the lamina propria (LP) and in the intraepithelial compartment and compared to controls.
Results: The patients suffered from watery diarrhea with few leukocytes rarely accompanied by blood. It began 4 to 14 days after therapy initiation and lasted on average 4.5 weeks. Colonoscopic appearance ranged from normal to a erythematous mucosa with multifocal ulceration. Upper endoscopies were normal. Histologically, a dense inflammatory expansion of LP was nearly always seen along the entire GI tract. Granulomas and features of chronicity were absent. Increased intraepithelial lymphocytes (IELs) were observed in all biopsies. Diffuse gastritis with apoptosis and focal glanditis were seen. Ileal villous blunting was noted as well as colonic cryptitis with apoptosis. IELs were more predominant in the colonic crypts than on the surface and consisted primarily of CD8 cells while the LP lymphocytes were a mixture of CD4 and CD8 cells. $FoxP3^+$ T_{reg} cells were greatly increased.
Conclusions: α-CLTA-4 mAb can be associated with a pan-enteritis. The changes are associated with an increase of T_{reg} in the mucosa, suggesting that instead of a systemic depletion, a redistribution of these cells to the gut occur possibly as a result of the enteric inflammation. These findings argue that the mechanism of α-CTLA-4 mAb induced mucosal changes involves the abrogation of CTLA-4's function on arresting T cell expansion, rather than through the depletion of T_{reg}.

561 Interobserver Variability in the Diagnosis of Hyperplastic and Serrated Colonic Polyps

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Background: Recently, certain types of "hyperplastic" (HP) appearing colonic polyps, traditionally considered benign, have been shown to have significant neoplastic potential and are termed sessile serrated adenomas (SSA). Some SSA resemble HP, whereas others show serrated adenoma (SA) or mixed hyperplastic/adenomatous (MHA) features. Reported rates of certain molecular aberrations in these lesions are quite variable; presumably due to interobserver variability in establishing diagnoses, although this has never been tested. The aim of this study was to determine the interobserver variability in interpretation of hyperplastic/serrated lesions of the colon with the ultimate goal of formulating reproducible criteria for these lesions.
Design: Routinely processed polypectomy samples from 42 hyperplastic/serrated lesions of the colon, initially diagnosed as HP (n = 18, including goblet cell, mucin depleted and microvesicular types), SSA (n = 9), SA (n = 7) and MHA polyps (n=8), were categorized separately by 8 GI pathologists into 1 of 5 diagnostic types [(HP, SSA, SA, conventional adenoma, MHA)] in a blinded fashion. Kappa statistics were used to determine interobserver variability.
Results: The overall level of interobserver agreement, for all lesions, was fair at best (k = 0.41, p<0.0001). Overall, 6 or more pathologists agreed on the type of polyp in only 22 (52%) of cases. All 8 pathologists agreed on only 5 (12%) cases. However, some lesions (HP and MHA) showed moderate levels of agreement (k = 0.57 and 0.50, respectively) compared to others [SSA: k = 0.21 and SA: k = 0.40)]. However, pathologists showed good agreement in distinguishing HP from other types of polyps (k=0.57, p<0.0001) and in distinguishing morphologically non-dysplastic, from dysplastic, lesions (HP or SSA vs. SA or MHA, k = 0.66, p<0.0001).
Conclusions: Interobserver agreement regarding diagnosis of hyperplastic/serrated polyps of the colon is only fair to good, even among GI pathologists with previous research experience in this field. Standardized and reproducible criteria are needed in order that future studies can define the biological features of these lesions in a consistent and reliable fashion.

562 MSI-High CIMP-High Colorectal Cancers Show Frequent Down-Regulation of Nuclear p27 (CDKN1B), While Non-MSI Non-CIMP-High Tumors Show Frequent Down-Regulation of p21 (CDKN1A)

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Background: p21 (*CDKN1A*/CIP1) and p27 (*CDKN1B*/KIP1) are cyclin-dependent kinase inhibitors, and play important roles in regulating the cell cycle. p21 is transcriptionally regulated by p53, while p27 is posttranslationally regulated by the ubiquitin-proteasome pathway. Microsatellite instability (MSI) and CpG island methylator phenotype (CIMP) represent two distinct phenotypes (molecular classifiers) in colorectal cancer, reflecting global cellular genomic and epigenomic alterations, respectively. However, no study to date has evaluated interrelationship between p27, p53, MSI and CIMP in colorectal cancer.
Design: We utilized 737 population-based samples of colorectal cancers from two large prospective cohort studies. By quantitative real-time PCR (MethyLight), we measured DNA methylation in five CIMP-specific gene promoters {*CACNA1G*, *CDKN2A* (p16), *CRABP1*, *MLH1* and *NEUROG1*}. We also assessed MSI status, expressions of p21, p27 and p53 by immunohistochemistry, and mutations in *KRAS* and *BRAF* by sequencing.
Results: CIMP-high (≥ 4/5 methylated promoters) was diagnosed in 118 (16%) of the 737 tumors. Loss of nuclear p27 expression (observed in 33% of all tumors) was significantly correlated with CIMP-high and MSI-H, and these associations were much more pronounced among p53-negative tumors than p53-positive tumors. In contrast, loss of p21 (observed in 50% of all tumors) was inversely associated with CIMP-high and MSI-H, regardless of p53 status. Then, we subclassified tumors according to MSI and CIMP status. p27 loss was most common in MSI-H CIMP-high tumors (62%=48/78, p<0.0001) and least common in non-MSI non-CIMP-high tumors (28%=152/544). p21 loss was most common in non-MSI non-CIMP-high tumors (58%=329/570, p<0.0001) and least common in MSI-H CIMP-high tumors (13%=11/85). Relations between *KRAS/BRAF/CDKN2A* (p16) and p21/p27 appeared to be secondary and mediated through CIMP status.
Conclusions: In colorectal cancer, down-regulation of p27 is positively correlated with CIMP-high and MSI-H, while down-regulation of p21 is inversely correlated with CIMP-high and MSI-H. Our data indicate differential associations of p21 loss and p27 loss with opposite molecular subtypes of colorectal cancer.

563 A Large Population-Based Colorectal Cancer Sample Shows Correlations of CpG Island Methylator Phenotype-Low (CIMP-Low) with Male Sex and *KRAS* Mutations

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Background: The CpG island methylator phenotype (CIMP or CIMP-high) with extensive promoter methylation is a distinct epigenetic subtype of colorectal cancer. However, no study has comprehensively examined biological significance of less extensive promoter methylation (designated as "CIMP-low") in colorectal cancer using a large number of samples and extended panel of methylation markers.
Design: Utilizing real-time PCR (MethyLight technology), we quantified DNA methylation in eight CIMP-specific gene promoters {*CACNA1G, CDKN2A (p16)*, *CRABP1, IGF2, MLH1*, *NEUROG1, RUNX3* and *SOCS1*} in 920 relatively unbiased, population-based colorectal cancer samples, obtained from two large prospective cohort studies. *MGMT* was excluded because of its relatively low sensitivity and specificity. We determined microsatellite instability (MSI) status using 10 microsatellite markers, sequenced the *KRAS* and *BRAF* genes, and assessed p53 by immunohistochemistry.
Results: CIMP-low (defined as 1/8-5/8 methylated promoters) colorectal cancers were significantly more common among men (42%=173/410 vs. 35%=180/510 in women, p=0.03) and among *KRAS*-mutated tumors (49%=158/325 vs. 34%=157/459 in *KRAS*/*BRAF* wild-type tumors, p=0.0003; 22%=25/113 in *BRAF*-mutated tumors, p<0.0001). In contrast, CIMP-high tumors (with ≥6/8 methylated promoters) were more common among women (18%=93/510 vs. 10%=43/410 in men, p=0.001) and among *BRAF*-mutated tumors (71%=80/113). CIMP-0 tumors (with 0/8 methylated promoters) did not show any sex predilection and were common in *KRAS/BRAF* wild-type tumors (58%=266/459). *KRAS* mutations were significantly more common in CIMP-low tumors (46%=158/342) than CIMP-high tumors (13%=17/135, p<0.0001) and CIMP-0 tumors (35%=150/426, p=0.002). The associations of CIMP-low tumors with male sex and *KRAS* mutations persisted after tumors were stratified by microsatellite instability (MSI) or p53 status. Regardless of MSI status, CIMP-low tumors showed a slight predilection to right colon, not as strongly as CIMP-high tumors.
Conclusions: A summary of associations in colorectal cancer was as follows: CIMP-0 with *KRAS/BRAF* wild-type (no sex predilection); CIMP-low with *KRAS* mutations and male; and CIMP-high with *BRAF* mutations and female. Our data raise the hypothesis that CIMP-low tumors may be biologically different from CIMP-high and CIMP-0 tumors.

564 Do Histopathologic Features of Colorectal Carcinoma Predict Microsatellite Instability (MSI) or CpG Island Methylator Phenotype (CIMP) or Both?

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Background: Extensive gene promoter methylation in colorectal cancer has been termed the CpG island methylator phenotype (CIMP). Most sporadic microsatellite instability-high (MSI-H) colorectal cancers are caused by *MLH1* promoter methylation in CIMP-high tumors. MSI has been associated with a variety of pathologic features. However, it is not clear whether those pathologic features are primarily associated with MSI or CIMP or both.

Design: Utilizing real-time PCR (MethyLight technology), we quantified DNA methylation in eight CIMP-specific gene promoters {*CACNA1G, CDKN2A (p16)*, *CRABP1, IGF2, MLH1*, *NEUROG1, RUNX3* and *SOCS1*} in 889 colorectal cancers obtained from two large prospective cohort studies. CIMP-high was defined as the presence of ≥6/8 methylated promoters, based on *KRAS* and *BRAF* mutation rates. We examined histopathologic features {mucinous/signet ring cell components, tumor border, differentiation, necrosis (non-intraglandular), sheeting, Crohn's-like lymphoid reaction, tumor infiltrating lymphocytes (TILs) and peritumoral lymphocytic reaction} while blinded from any other laboratory data.

Results: CIMP-high was diagnosed in 15% (=134/889) of tumors. We classified the 889 colorectal cancers into four subtypes, namely, MSI-H CIMP-high (N=93), MSI-H non-CIMP-high (N=38), non-MSI-H CIMP-high (N=41), and non-MSI-H non-CIMP-high tumors (N=717). The presence of >=50% mucinous component was more common in MSI-H tumors (20-24%, p=0.0008) than non-MSI-H tumors (6.3-12%) regardless of CIMP status. In contrast, poor differentiation was more common in CIMP-high tumors (17-36%, p=0.009) than non-CIMP-high tumors (5.2-5.4%) regardless of MSI status. The presence of any signet ring cell component also showed a similar trend (17-20% in CIMP-high vs. 4.5-7.9% in non-CIMP-high tumors). For necrosis, sheeting, and Crohn's-like and other lymphocytic reactions, MSI-H and CIMP-high exhibited synergistic effects of increasing the frequencies of these features.

Conclusions: Mucinous tumors are associated primarily with MSI, while poor differentiation and signet ring cells are associated with CIMP. In contrast, Crohn's-like reaction, TILs, necrosis, and sheeting are associated with both CIMP and MSI. Both MSI and CIMP appear to play roles in the pathogenesis of specific morphologic patterns. Our data support a predictive power of histopathology for molecular classification of colorectal cancer.

565 Appendiceal Cystadenoma/Dysplasia in IBD: Fortuitous Association or Neoplastic Complication?

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Background: Cystadenomas and cystadenocarcinomas are relatively uncommon appendiceal neoplasms. Although an association between these entities and sporadic colorectal cancer has been proposed, there is thus far little evidence of an association with diseases that predispose to colorectal cancer such as familial polyposis and HNPCC. Considering the frequent appendiceal involvement in inflammatory bowel diseases (IBD) and the known predisposition of patients with IBD to colorectal neoplasia, we investigated whether a relationship exists among epithelial (non-carcinoid) appendiceal neoplasia (AN), IBD, and colorectal neoplasia.

Design: Our computerized pathology database was searched for all colectomy specimens with appendices processed for histology during a 4-year interval. Cases with AN, i.e., dysplasia, cystadenoma or cystadenocarcinoma, were recorded and assigned to one of 4 categories: IBD, non-IBD, with and without synchronous colorectal neoplasia (CRN), i.e., adenocarcinoma, adenomatous polyps, recent endoscopically resected malignant polyps, or dysplasia. Cases of AN in appendectomy specimens during the same 4-year interval were recorded separately.

Results: Ten cases of AN were associated with IBD. Nine were discovered incidentally among 691 colectomy specimens (1.3%; 6 ulcerative colitis, 3 Crohn's disease), and comprised 7 mucinous cystadenomas and 2 cases of non-cystic dysplasia. One additional case was diagnosed in an appendectomy specimen from a patient with known ulcerative colitis. All 10 cases were characterized by low-grade dysplasia. None were accompanied by contiguous cecal dysplasia. The prevalence of AN among IBD colectomies with synchronous CRN was 5 times that of IBD colectomies without CRN, although the difference fell short of statistical significance (4/98 [4%] vs. 5/593 [0.8%]; p=0.07), and 10 times that of non-IBD colectomies as a whole (4/98 [4%] vs. 2/512 [0.4%]; p=0.008). However, it was not significantly higher than in non-IBD colectomy specimens with CRN (4/98 [4%] *vs.* 2/147 [1.4%]; p=0.22).

Conclusions: IBD predisposes to AN, especially in cases with synchronous colorectal carcinoma and/or dysplasia, suggesting that AN is part of the spectrum of neoplastic complications of IBD.

566 Gastric Foveolar (Type II) Dysplasia Frequently Arises from a Background Mucosa with a Distinctive form of Intestinal Metaplasia

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Background: Intestinal metaplasia (IM) has traditionally been classified into incomplete and complete types based on morphological and histochemical features. However, recent MUC stains and CD10 immunohistochemical studies suggest that IM can also be subdivided into a *complete intestinal mucin phenotype* (Muc2+,CD10+,Muc5ac-) and an a phenotype retaining expression of gastric type mucin (Muc2+, CD10+/,Muc5ac+), being termed as a *mixed gastric and intestinal type of IM (mixed GI IM)*. However, the relevance of these two phenotypes of IM to the morphological type of gastric epithelial dysplasia (GED), i.e., adenomatous vs. foveolar (type II), has not been evaluated thus far.

Design: We evaluated 69 cases of GED obtained by endoscopic mucosal resection for expression of MUC2, MUC5AC, MUC6 and CD10. The immunoreactivity for these markers was evaluated both in dysplastic mucosa as well as in the immediately adjacent IM present in the background gastric mucosa. Expression of each marker in ≥ 10% of each examined lesion was considered positive. GED was classified as adenomatous, foveolar, or hybrid (showing features of both types), based on routine histologic evaluation combined with the results of CDX2, CD10 and MUC stains.

Results: Intestinal metaplasia was identified in the background mucosa in 15/15 (100%) cases of foveolar (type II) dysplasia, in 23/23 (100%) cases of hybrid type GED, and 27/31 (87.1%) cases of adenomatous type GED. When compared to adenomatous type GED (10/31, 32.3%), foveolar (12/15, 80.0%) and hybrid types (19/23, 82.6%) of GED were associated more often with IM showing a retained expression of gastric type mucin (mixed GI IM) (p=0.004, p=0.001, respectively). In contrast, adenomatous type (22/31, 71.0%) of GED was significantly associated with a complete intestinal mucin phenotype (CD10+) than foveolar (5/15, 33.3%) and hybrid type (7/23, 30.4%) of GED (p=0.015, p=0.003, respectively).

Conclusions: Our findings suggest that the two morphologically and immunophenotypically distinctive types of GED arise in a background of immunophenotypically different type of IM. The foveolar and hybrid types of GED are significantly associated with IM showing retained expression of gastric type (Muc5ac+) mucin whereas the IM in patients with adenomatous type GED is typically of a completely intestinal mucin phenotype (CD10+, Muc5ac-).

567 Immunohistochemical Expression of MUC Stains, CD10 and CDX2 in Morphological Variants of Gastric Epithelial Dysplasia with Emphasis on Type II (Foveolar Type) Dysplasia

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Background: Gastric epithelial dysplasia (GED) can be morphologically categorized into adenomatous (or intestinal) and foveolar (or gastric / type II) types. It has been proposed by some that the foveolar subtype of GED is almost always low grade when it is detected as a polypoid lesion. Further, while no distinct genetic differences have been demonstrated between these two subtypes thus far, the immunophenotypic expression of CDX2, CD10 and MUC stains has not been simultaneously evaluated in this context previously.

Design: Endoscopic mucosal resections from 69 cases of GED were evaluated systematically for immunohistochemical expression of various MUC stains, CD10 and CDX2 and the findings were correlated with morphological categorization and with several clinico-pathological parameters including endoscopic appearance, size and grade. GED was classified as adenomatous, foveolar, or hybrid (showing features of both types), based on routine histologic evaluation. CDX2, MUC2, MUC5AC, MUC6, as well as CD10 immunostains were performed in all cases.

Results: An adenomatous morphology was seen in 31/69 cases (45.0%), hybrid in 23/69 (33.3%) and a "pure" foveolar subtype was seen in 15/69 cases (21.7%). Foveolar type GED was associated more often with a depressed/flat appearance on endoscopy (p=0.075), smaller size (p=0.026) and a high grade morphology (p=0.046) when compared to the adenomatous type. MUC studies confirmed the histologic stratification: the foveolar and hybrid types showed more MUC5AC (p=0.001 and 0.001, respectively) and less CD10 (p=0.019 and 0.016, respectively) expression compared to the adenomatous type. Conversely, extensive CDX2 expression (>50% positivity) was associated almost exclusively with an adenomatous type (p=0.0001).

Conclusions: The immunophenotypic expression patterns of CDX2, CD10 and MUC stains confirms the presence of two epithelial phenotypes of GED: intestinal and gastric/foveolar type. We also identified a hybrid variant that shows admixtures of the both subtypes in varying proportions. The "pure" gastric foveolar type of GED as defined by both morphological and immunophenotypic features may have distinct clinicopathologic characteristics including higher grade. The clinical and biologic significance of the three subtypes (including hybrid lesions) needs to be investigated in future studies.

568 Marked Global DNA Hypomethylation in the Non-Neoplastic Mucosa from Gastric Cancer Patient and Its Association with Multi-Step Gastric Carcinogenesis

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Background: It is known that cancer cells generally harbor global hypomethylation and promoter hypermethylation concurrently. Especially, DNA hypomethylation has been proposed recently to play a role in cancer gene activation and genomic instability. However, the role of hypomethylation in carcinogenesis is unclear. To understand of the role of hypomethylation, assessment of hypomethylation levels in precancerous lesion would be needed.

Design: Formalin-fixed representative samples of chronic gastritis (n=45), intestinal metaplasia (n=45), tubular adenomas (n=35), gastric adenocarcinomas (n=60) and normal mucosa from neoplastic lesion (n=25) were used in this study. The methylation levels of LINE-1 and ALU repetitive sequences in genomic DNA were evaluated by combinated bisulfite restriction analysis.

Results: A graduated decrease in methylation levels was found over the range extending from chronic gastritis, to gastric adenomas, and to gastric carcinomas (18.8, 17.5 and 15.2 percentage of Alu, 54.6, 52.1 and 36.8 percentage of LINE-1, respectively). No differences existed between intestinal metaplasia and gastric adenomas in methylation levels (17.7 and 17.5 percentage of Alu, 53.4 and 52.1 percentage of LINE-1,

respectively). Also, we found that the methylation levels of Alu and LINE-1 in the non-neoplastic gastric mucosa from gastric cancer patients were much lower than the respective one in the non-neoplastic mucosa or gastric adenoma from patients without gastric cancer.
Conclusions: Our findings suggest that global DNA hypomethylation occurs early in the course of multi-step gastric carcinogenesis and gastric cancer arises in the background of gastric mucosa with marked global genomic hypomethylation.

569 Eosinophilic Esophagitis: Study of Pathogenesis, Stem Cell Proliferation and Biomarker Eotaxin-3

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Background: The etiopathogenesis of eosinophilic esophagitis (EE) is thought to be primarily related to allergy. Histologically it is manifested by increased intramucosal eosinophils, basal cell hyperplasia (BCH), spongiosis, papillary height elongation and lamina propria fibrosis. It has been speculated that this response is due to interplay of immune-mediated factors such as chemokine Eotaxin-3 which exhibits selective chemotactic activity for eosinophils. However, the exact pathogenetic sequence of events remains undetermined.
Design: One-hundred and forty six cases of eosinophilic esophagitis from archived material were reviewed and following variables were documented: age, no. of biopsies / procedure, location (proximal/distal), BCH (≤ 25%- normal, mild: 26-50%, moderate: 51-75%, severe >75%), spongiosis and number of eosinophils (≥15/hpf was diagnostic). Immunostains for p63, Ki-67 and Eotaxin-3 were performed with appropriate positive and negative controls.We systematically analyzed a) the relationship of eosinophils with cell injury, b) the relationship of stem cell marker p63 and proliferative marker Ki-67 with degree of basal cell hyperplasia and c) role of activation marker Eotaxin-3, to establish a sequence of events from inflammation to repair.
Results: A total of 214 biopsies were reviewed (146 patients, 103 males, 43 females). Eighty-five patients had biopsy from one site while 61 patients had biopsies from 2 or more sites. While biopsies from distal end showed significantly more BCH compared to those from proximal end (p=0.002), spongiosis did not show any such correlation. In the 51 cases stained for p63 and Ki-67, severity of BCH was significantly associated with higher p63 (0.034) and Ki-67 (p=0.001) expression. Degree of spongiosis correlated with increased proliferation (p=0.03) and increasing number of eosinophils (p= 0.0001). When compared to cases of reflux esophagitis (n=20, all from distal end), more BCH, higher p63 expression and stronger and diffuse Eotaxin-3 expression was identified in cases of EE (p=0.001, n=27, from distal esophagus).
Conclusions: We have demonstrated that active intraepithelial eosinophils are associated with epithelial injury (spongiosis) and stem cell/ progenitor cell proliferation. High expression of p63 and Ki-67 indicate that stem cells actively participate in the repair process of eosinophilic esophagitis. Eotaxin-3 expression may serve as a biomarker for differentiating EE from reflux esophagitis.

570 p16 Expression Is Associated with Familial Status in MLH1-Deficient Colorectal Carcinomas

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Background: Colorectal carcinoma (CRC) with loss of MLH1 expression can be sporadic or familial. Promoter hypermethylation of several genes and BRAF mutations are known to distinguish hereditary non-polyposis colon cancer (HNPCC) from sporadic MSI CRC. Sporadic cases show promoter hypermethylation of MLH1, which are frequently associated with hypermethylation of other genes and regions, such as p16 and MINTs. Therefore, there are some genetic differences between sporadic and familial MSI tumors, that could be associated with different immunohistochemical profile. The aim of our study was to evaluate the clinical significance of p16 protein expression in a series of MLH1-deficient CRC.
Design: We selected 67 CRC with loss of MLH1 (BD Pharmingen) protein expression. 15 (22%) were familial, defined by the presence of germline-mutation and/or meeting Amsterdam II criteria. We performed a microsatellite instability (MSI) status analysis using BAT26, and immunohistochemistry for mismatch-repair proteins (MLH1, MSH2, MSH6, PMS2). BRAF-V600E mutation detection was performed in a ABIPRISM 7500 based on the design of two TaqMan probes labelled with a different fluorescent tag specific for the wild-type and the mutant allele. P16 (Santa Cruz) immunostaining was classified as negative (<1% of positive nuclei) or positive.
Results: Loss of p16 immunohistochemical expression was observed in 29% (15/52) sporadic and in 0% (0/15) MLH1-deficient HNPCC cases (p=0,018). BRAF mutations were observed in 43% of sporadic and in 0% MLH1-deficient HNPCC cases (p=0,005). No correlation was found between p16 expression and BRAF gene status. For the detection of sporadic cases, the positive predictive value of p16 expression was 100% and the negative predictive value was 29%. The sensitivity and specificity were 29% and 100%, respectively.
Conclusions: Our results suggest that MLH1-deficient CRCs with loss of p16 are sporadic, as well as tumors with BRAF mutations. Therefore, in these cases the analysis of germline mutation can be avoided.

571 The Clinical Significance of Focal Enhanced Gastritis and Isolated Terminal Ileitis in Adult Patients

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Background: Isolated chronic terminal ileitis, accompanied by no or very mild chronic colitis, is not an uncommon finding present in biopsy specimen for chronic diarrhea evaluation. A spectrum of entities should be included in the differential diagnosis; the most common being Crohn's disease (CD) and drug-induced enterocolitis, especially due to non-steroid anti-inflammatory drugs (NSAIDs). At present, there is no gold standard test to establish the diagnosis of CD for certainty. Focal enhanced or active gastritis (FEG) was considered as a good CD predictor in high prevalence CD population, but not in the general clinical setting. We would like to evaluate if FEG is a good marker for CD in patients with isolated terminal ileitis.
Design: Forty-six consecutive cases of isolated terminal ileitis with concurrent stomach biopsy were retrieved from our archive. Patients' ages were between 18 and 55 years. Biopsies were reviewed and included in the study provided the patient had no previous history of inflammatory bowel disease, and the biopsy showed terminal chronic ileitis without obvious evidence of chronic colitis. Cases with epithelioid granulomas not associated with cryptitis or crypt rupture were excluded from the study. The diagnostic criteria of chronic ileitis included crypt distortion and inflammation, plasmacytosis in laminar propria, ulceration, and/or pyloric gland metaplasia. Clinical follow-up was obtained from patient records.
Results: Twenty five of these 46 cases developed CD, confirmed by follow-up clinical manifestations and/or biopsies. The stomach biopsies of these patients presented with a spectrum of histological findings, including FEG, chronic gastritis with or without Helicobacter Pylori organisms, chemical gastropathy and normal. The comparison of stomach biopsy findings between CD and non-CD patients is shown in the table.

Diagnosis	CD (n=25)	Non-CD (n=21)
FEG	9	1
Chemical Gastropathy	2	9
Chronic Gastritis w/o H.P.	10	6
Chronic Gastritis with H.P.	0	3
Normal	4	3

Conclusions: Focal enhanced gastritis (FEG) is commonly present in Crohn's disease patients (36%) (P<0.01), while chemical gastropathy is prevalent in non-Crohn's disease patients (45%) (P<0.01). The positive predictive value (PPV) of FEG for the diagnosis of CD is 0.9, while PPV of chemical gastropathy for non-CD is 0.8. Therefore, the presence of FEG in an adult with isolated chronic terminal ileitis indicates the patient has Crohn's disease.

572 The Expression of Mismatch Repair Proteins, O(6)-Methylguanine-DNA Methyltransferase (MGMT), β-Catenin and p53 in Appendiceal Adenocarcinomas

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Background: Microsatellite instability in colorectal carcinoma (CRC) can be due to mutations in the mismatch repair (MMR) genes (Lynch syndrome) or methylation of the *MLH1* promoter resulting in loss of immunostaining for the associated protein. Promoter methylation can also silence immunoreactivity for MGMT and is seen in some CRCs. CRCs due to DNA methylation are associated with an improved prognosis and tend to show a serrated and mucinous morphology. Because many appendiceal tumors are mucinous, arise from serrated lesions and may show prolonged survival, we evaluated mucinous and non-mucinous appendiceal tumors to determine whether any are associated with loss of MMR or MGMT protein expression. β-catenin and p53 were also assessed to further investigate tumorigenesis in appendiceal tumors.
Design: Thirty-nine appendiceal adenocarcinomas were retrieved from the archival files. All cases were reviewed and graded as tumors of low malignant potential (LMP), mucinous adenocarcinoma (MA), or adenocarcinoma (Ad) according to Pai et al., Adv Anat Pathol 12:291-311, 2005. Tissue cores from formalin-fixed paraffin embedded donor blocks (4 cores per block) were arranged to create a tissue microarray of 2.0 mm cores and immunostained with antibodies against MMR proteins (MLH1, MSH2, MSH6, PMS2), MGMT, β-catenin and p53. Greater than 1% of tumor cells staining for the MMR proteins and greater than 5% of tumor cells staining for β-catenin and p53 were considered positive. MGMT was interpreted as having loss of expression when there was staining in <10% of tumor cells.
Results: 15 cases were classified as LMP, 13 MA, and 11 Ad. All cases showed intact expression of the 4 MMR proteins and were immunoreactive with β-catenin. Overexpression of p53 was identified in 1 of 15 (6.7%) LMP, 2 of 13 (15.4%) MA, and 5 of 11 (45.5%) Ad. Loss of expression of MGMT was observed in 7 of 15 (46.7%) LMP, 5 of 13 (38.5%) MA, and 6 of 11 (54.5%) Ad.
Conclusions: Appendiceal adenocarcinomas do not appear to arise via MMR defects but β-catenin, p53 and loss of MGMT may play a role in tumorigenesis. The mucinous tumors showed fewer p53 mutations compared to the non-mucinous, however, both showed similar frequencies of MMR and MGMT protein loss and β-catenin expression. Although slight differences in protein expression patterns were found, additional studies are warranted to further investigate the cancer pathway in mucinous and non-mucinous appendiceal adenocarcinomas.

573 The Significance of Pyloric Gland Metaplasia in Pouch Biopsies in Patients with Presumed Ulcerative Colitis or Inderminate Colitis Treated by Ileal Pouch Anal Anastamosis

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Background: Ileal pouch anal anastamosis (IPAA) is a continence restoring procedure after colectomy in patients with ulcerative colitis (UC) or indeterminate colitis (IC). IPAA is generally contraindicated in patients with known Crohn's disease (CD) due to high disease recurrence rate and subsequent pouch failures. Some patients receiving IPAA originally diagnosed with UC or IC eventually are proven to have CD. In the clinical setting of inflammatory bowel disease, some authors have suggested that pyloric gland metaplasia (PGM) is a specific feature of CD. This study sought to determine the significance of PGM in pouch biopsies in patients with presumed UC/IC treated with IPAA.

Design: Patients were identified from an institutional IBD pouch database. Pouch biopsy pathology reports were reviewed in order to identify reports that specified the presence or absence of PGM. In all, 117 cases were found, and all of these pouch biopsies were re-reviewed. PGM was confirmed in 39 cases, while the non-PGM group included 78 cases. Clinical records were reviewed to determine if the ultimate clinical diagnosis remained as UC/IC or if it changed to CD.
Results: Overall, 31/117 (26%) patients were clinically considered to have CD as of their last follow-up visit. Of these, 17/39 (44%) patients with PGM on pouch biopsy were considered to have CD. In contrast, only 14/78 patients (18%) without PGM developed CD (p=0.003). The positive and negative predictive values of PGM on pouch biopsy for a clinical diagnosis of CD were 44% and 82%, respectively. Granulomas were not identified in any of the biopsy specimens.
Conclusions: There was a high incidence of CD of the pouch in this patient population. The presence of PGM correlated with an increased frequency of a clinical diagnosis of CD of the pouch. Although not entirely specific, the presence of PGM in pouch biopsy specimens should be specifically sought and reported, as it is associated with a clinical diagnosis of CD in patients who undergo IPAA for a presumed diagnosis of UC/IC.

574 Lymphocytic Esophagitis: What It Is and What It Is Not

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Background: Lymphocytic esophagitis (LE) is a newly described histologic entity of unknown cause. It is characterized by prominent intraepithelial lymphocytes (IEL) and spongiosis, resembling contact dermatitis. Rubio defined the criteria for LE as high numbers of IEL in peripapillary fields (mean 55.1 /270 μm^2), fewer IEL in interpapillary fields (mean 20.3/270 μm^2), and rare granulocytes. LE is reported to occur in young patients, and to be associated with Crohn's disease, compared to patients with other forms of acute esophagitis (Rubio, 2006).
Design: We reviewed the medical records of 42 patients with LE whose biopsies satisfied the Rubio criteria. The biopsies were divided into those with severe LE—IEL in interpapillary and peripapillary fields, and mild LE—IEL in peripapillary fields only. We compared the LE patients with a control group of 34 consecutive patients having esophageal biopsies for any purpose other than Barrett's surveillance, 26 of whom had normal biopsies. This is different from control patients in the Rubio study, all of whom had some form of esophagitis.
Results: There is no significant difference in ages of LE patients (2-92, mean 47) and controls (10-70, mean 42). Four (9%) of LE patients had Crohn's disease, compared with 1 control (2% of all controls, 4% of normal controls), an insignificant difference. Fourteen (33%) had a form of allergy (seasonal allergies, asthma, allergic skin conditions); 17 (40%) had GERD symptoms; 6 (14%) had *H pylori* gastritis and 17 (40%) presented with dysphagia. For none of the clinical features was there a significant difference between LE and controls, except GERD symptoms in intense LE patients(10/29, 34%), significantly different from normal controls (18/26, 69%). No LE patient had celiac disease. Review of the medical records disclosed no medications common in the LE patients.
Conclusions: Patients with lymphocytic esophagitis are no more likely than other patients having esophageal biopsies, including those with normal biopsies, to have Crohn's disease, nor is this population younger, findings different from the only other published study. The only significant difference between the LE and controls was that nearly twice as many control patients with normal biopsies 18/26 (69%) had symptoms of GERD, compared to 10/29 (34%) severe LE patients. LE patients were no more likely than controls to suffer from allergies, *H pylori* gastritis, dysphagia, or celiac disease. No medication was common among LE patients. Thus, we have found no association between LE and any specific clinical condition.

575 Colorectal Adenomas with Mixed Conventional and Serrated Adenomatous Features: A Clinicopathologic and Immunophenotypic Study of 15 Cases

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Background: There is evidence to suggest that conventional (tubular, tubulovillous, villous) adenomas (CA) and serrated adenomas (SA) may progress to carcinoma via different molecular pathways. Rarely, we have noted that some colonic adenomas show a mixture of CA (pencil-shaped hyperchromatic nuclei, mucinous cytoplasm) and SA (oval shaped nuclei, open chromatin pattern, eosinophilic cytoplasm, luminal serration) features in the same polyp, but the significance of this finding has never been evaluated. The aim of this study was to evaluate the clinicopathologic and immunophenotypic features of colonic adenomas that showed mixed CA and SA cytologic features.
Design: Routinely processed tissue sections of 15 polyps from 15 patients were retrieved by a search of 450 consecutive colonic adenomas (prevalence: 3.3%) and evaluated for a variety of pathologic and immunohistochemical (loss of hMLH-1, hMSH-2, and MGMT, presence of nuclear b-catenin and degree of Ki-67 staining) features. Staining was compared between CA and SA foci within the same polyps. 100 consecutive CA, from 79 patients, without SA cytologic features, were used as controls for the clinical and pathologic data.
Results: Of the 15 study patients (M/F ratio: 8/7, mean age: 63 years, range 51-81 years $p>0.05$ vs CA), 3 and 7, respectively, also had either previous (n=3) or concurrent (n=7) hyperplastic polyps or CA, respectively. None had associated SA or sessile serrated adenomas. Their mean size was 1.7 cm (range; 0.4-3.8cm), which was significantly higher than control CA (mean: 0.5 cm, $p<0.05$). The distribution of polyps in the colon and degree of dysplasia was statistically similar. Microscopically, the CA portion of the polyps consisted of conventional tubular (n=9) or tubulovillous (n=6) units containing cells with typical CA morphology. SA foci (which formed less than 5% of the surface area of the polyps in all cases) were located either in separate crypts, or within the same crypts that showed CA cytologic features. 93% of SA foci showed decreased Ki 67 staining, but none showed loss of hMLH-1, hMSH-2 or MGMT in either the CA or SA component. However, 7/15 (47%) polyps showed nuclear b-catenin in CA foci, but in none (0%) of the SA foci ($p<0.01$).
Conclusions: Adenomas with mixed CA and SA cytologic features are rare lesions (prevalence: 3.3%) that provide morphologic and molecular evidence that multiple molecular pathways may be involved in the pathogenesis of some colonic adenomas.

576 A Distinct Spectrum of Pathology Is Associated with KRAS Mutations in the Serrated Polyp Neoplasia Pathway

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Background: The serrated polyp neoplasia pathway is an alternative pathway to colorectal carcinoma most frequently instigated by BRAF mutations and characterized by an MSI endpoint carcinoma. Our aim is to identify the spectrum of pathology with a demonstrable serrated histogenesis that exhibits KRAS mutations and test the hypothesis that it is distinct from those with BRAF mutations.
Design: Serrated polyps were initially selected by random sampling of hyperplastic polyps categorized by proximal vs. distal location and small (<5mm) vs. large (> 5mm), assayed for BRAF and KRAS and classified blindly using criteria based on those of Torlakovic et al as Goblet Cell (GCSP) Microvesicular (MVSP) Serrated Polyps & Serrated Polyp with Abnormal Proliferation (SPAP, syn: Sessile Serrated Adenoma SSA). Following analysis of these cases (n = 81) an additional 24 GCSPs and 27 MVSPs that showed typical histological criteria, were assayed to validate the initial histological associations. Assays for both mutations were also performed on sequentially accessioned Serrated Adenomas (SAs) identified from department files.
Results: Findings in a total of 173 serrated polyps are presented in table 1. BRAF and KRAS mutations were mutually exclusive in all categories. 14/25 of SAs with BRAF mutations, 3/5 null and 0/11 with KRAS mutations had contiguous SPAPs. Retrospective audit of the 7 of 94 MVSPs(5) and SPAP(2) with KRAS mutations indicated misclassification or possible contamination by contiguous GCSP in 2/2 SPAPs and 4/5 MVSPs. Conversely the test sample of MVSPs and GCSPs yielded no example of KRAS mutation among 27 MVSPs, and only one BRAF mutation of 24 GCSPs.
Conclusions: While KRAS is a frequent instigating mutation of self-limited GCSPs it is rarely if ever identified in SPAP/SSAs although found frequently (27%) in SAs. We postulate that KRAS mutations develop "late" in null MVSPs causing their direct (i.e. not via SPAP/SSA) transformation to SAs.

Table 1, KRAS & BRAF Mutations By SP Histological Category

SP Category	KRAS Mut. (%)	Null (%)	BRAF Mut. (%)
GCSP (n=38)	19(50.0)	15(39.5)	4(10.5)
MVSP (n=65)	5(7.7)	11(16.9)	49(75.4)
SPAP (n=29)	2(6.9)	3(10.3)	24(82.8)
SA (n=41)	11(26.8)	5(12.2)	25(61.0)

Table 2, Fisher's Exact Test

Comparison	KRAS Mut., p	BRAF Mut., p
GCSP vs. MVSP	<0.0001	<0.0001
GCSP vs. SPAP	0.002	<0.0001

577 Prophylactic Total Gastrectomy in Hereditary Diffuse Gastric Cancer: Pathologic Findings with New Implications for Endoscopic Screening

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Background: Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant cancer susceptibility syndrome caused by germline E-cadherin (CDH1) mutations in 40% of cases with a high degree of penetrance. Screening endoscopy has not been useful in identifying early cancer, in part due to conflicting data concerning site(s) of involvement in the stomach.
Design: Prophylactic total gastrectomy (PTG) specimens from 7 asymptomatic individuals with germline mutations in the CDH1 gene (2 different pedigrees) and negative endoscopic screening were studied using a sequential serial sectioning protocol with submission of the entire stomach for histologic analysis. The presence, size, and distribution of signet ring cell clusters were determined for each section and geographic maps of the invasive foci were constructed and compared to gastrectomy specimens from patients with germline E-cadherin mutation and symptomatic gastric cancer.
Results: All PTG specimens were macroscopically normal. All but one contained multiple foci (mean, 9.1) of microscopic intramucosal signet ring cell carcinoma confined to the superficial half of the gastric mucosa; no invasion of submucosa was identified. In situ carcinoma was present in 3/7 cases. The majority of signet ring foci were located within the proximal 1/3 of the stomach, all within corporeal-type mucosa. The number and size of foci were not related to age or gender. Stomachs from the symptomatic group exhibited similar histologic features, with additional infiltrative foci that extended well beyond the superficial mucosa. One PTG specimen had diffuse, superficial lymphoplasmacytic inflammation; no other inflammatory foci or evidence of host response was present.

Sex	Age	# Foci	Largest (mm)	Proximal 1/3 (%)	Mid 1/3 (%)	Distal 1/3 (%)
F	53	17	1.5	53	12	29
F	52	11	2.5	55	18	27
F	55	8	3	75	25	0
F	50	12	1.5	100	0	0
M	56	2	1	50	0	50
M	51	13	1.5	69	0	31
F	42	1	0.25	100	0	0

Conclusions: Superficial intramucosal signet ring carcinoma, although widespread, is predominantly located in the proximal 1/3 of the stomach in patients in this series. The observed site predilection, which may be due to specific mutational and/or environmental factors, suggests a possible role for geographically targeted endoscopic surveillance biopsy in patients who elect to delay surgical intervention.

578 KIT Gene Molecular Analysis Distinguishes Multiple Primary GIST from Metastatic Disease

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Background: The occurrence of multiple primary GIST (mpGIST) has been reported exclusively in children and in NF1 patients.However,sometimes GIST patients present at onset with double lesions,raising the problem of the differential between metastatic disease and mpGIST originating either from gastrointestinal tube or peritoneum.Small size and lack of mitotic activity might help in distinguishing mpGIST,unless we deal with simultaneous malignancy.Molecular analysis of KIT gene might represent an additional important tool to discriminate among these 2 possibilities,allowing proper therapy.

Design: From a series of 200 GIST from 2 institutions,we selected 4 cases of non NF1 adult GIST patients,presenting double lesions at onset and considered as metastatic. DNA was extracted from paraffin-embedded tissue of both the nodules of each patients and KIT exons 11,9,13 and 17 were sequenced.

Results: Patients' age ranged from 48 to 82 years. 3 patients had a dominant small intestinal nodule and a peritoneal nodule.1 patient had a gastric nodule and a small intestinal nodule of comparable size.In 2 cases the mitoses were more than 10/50HPF and in 2 cases were less than 5/50HPF.No difference in mitotic rate and morphology was seen between the 2 nodules in each case.In 3 cases,no difference in the molecular features was detected between the nodules from the same patient,confirming the metastatic nature of the disease.In contrast,in 1 case,different KIT mutations were found,suggesting the occurrence of 2 synchronous GIST in this patient.Interestingly,in 1 nodule,2 mutations were detected.See table 1.

Conclusions: 1.Molecular analysis proves the existence of exceptional cases of mpGIST in adult non NF1 patients. 2.Since discrimination between metastatic disease at onset and mpGIST impacts over therapeutic strategies,it might be useful also in the routine practice to perform molecular analysis of both nodules in cases of double GIST.That might be particularly important in cases,in which the nodules have a comparable size and lack mitotic activity.

Table 1

CASES	SITE	SIZE (cm)	MIT/50HPF	MUTATIONS
1A	small bowel	10	20	Ex 11 W557G
1B	peritoneum	1.5	17	Ex 11 W557G
2A	small bowel	8	35	Ex 9 ins 502AY
2B	peritoneum	2	33	Ex 9 ins 502AY
3A	small bowel	3	1	absent
3B	peritoneum	2.3	1	absent
4A	stomach	6.2	2	Ex 9 del 29 nt with stop cod at 480
				Ex 11 W557G
4B	small bowel	6	1	Ex 11 VQWKVVEEINGNNYVYI 555-572del

579 Wnt-Signaling Pathway Proteins in Ampullary Carcinoma: A Tissue Microarray Analysis

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Background: Aberrant activation of Wnt signaling pathway has been recognized as one of the key pathways in the carcinogenesis of various tumors, particularly colon cancer. Recently, much interest has focused on different areas of the Wnt pathway with possible applications in cancer diagnosis, staging and therapy. Many alterations have been described, but β-catenin appears to be one of the most important proteins involved in this pathway. The aim of this study is to explore the contribution of this pathway in pathogenesis of carcinoma of the ampulla of Vater.

Design: The role of Wnt-signalling pathway proteins (adenomatous polyposis coli (APC), β-catenin, glycogen synthase (GSK-α), c-myc, E-cadherin and cyclin D1) in the pathogenesis of 39 cases of ampullary carcinoma was examined by tissue microarray analysis (TMA). All relevant clinicopathologic information was obtained and compared with the immunohistochemical findings.

Results: There were 25 males and 14 females with mean age of 66 years (range 37-87) composed of 7 T1, 16 T2 and 16 T3 tumors. Lymph node metastasis was noted in 17 cases (2 T1, 7 T2, 8 T3). Immunohistochemistry demonstrated nuclear b-catenin staining in 10 cases (25.6%). Eight were in males and 7 cases had lymph node involvement. Cyclin-D1 showed diffuse (greater than 75% of the tumor) nuclear staining in 31/39 cases (79.4%) and focal (25-50%) staining in the remaining 8 cases. Seven of 10 cases with nuclear localization of β-catenin exhibited diffuse cyclin-D1 overexpression and normal APC expression. APC expression was present in all cases and GSK-α positivity was noted in 38/39 cases. Membrane staining for E-cadherin was present in all cases ; in addition there was nuclear E-cadherin staining in 5/39 (12.8%), 2 of which also showed nuclear localization of b-catenin. Nuclear staining for c-myc was detected in all cases. Aberrant expression of β-catenin, a key component of the Wnt pathway was detected in 10/39 (25.6%) of cases in this study and correlates with advanced tumours (70% of them were stage III disease).

Conclusions: The above data suggest that while β-catenin is abnormal in a 25% of ampullary carcinomas, it is most likely due to mutations of the β-*catenin* gene instead of an abnormality of APC protein. Thus, the canonical wnt pathway does not appear to be involved in the pathogenesis of ampullary carcinomas via the APC/ β-catenin axis.

580 Molecular Analysis of Adenocarcinomas Involving the Periampullary Region

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Background: Histologic separation of adenocarcinomas arising in the periampullary region including the ampulla of Vater, head of pancreas and common bile duct (CBD) may be challenging since they can have similar histology and clinical presentation. Moreover, these tumors frequently metastasize, with no reliable immunohistochemical markers to identify the specific primary site with prognostic implications. In this study we investigated the utility of molecular markers, including K-ras mutations as aid to differentiate carcinomas in periampullary region.

Design: We examined the genetic alterations at 18 polymorphic DNA markers in the DNA tumors of 60 patients including 11 duodenal, 22 ampulla of Vater, 10 CBD and 17 pancreatic adenocarcinomas. The tumor tissue was microdissected from paraffin embedded biopsies. Mutations were quantitatively determined to detect K-ras point mutation and LOH for a broad panel of 18 markers.

Results: K-ras point mutation was observed in 13/17 (76%) of pancreatic adenocarcinoma, and was only seen in 3/22 (14%) ampulla of Vater adenocarcinomas. None of duodenal or bile ductal carcinomas showed K-ras mutation. 1p mutation was identified in 23/41 of duodenal, ampulla of vater and CBD carcinomas, and in only 5/17 cases of pancreatic adenocarcinoma, with significant correlation ($P<0.05$). Other markers were identified in various tumors with different percentages. The sensitivity, specificity, PPV and NPV of K-ras for pancreatic adenocarcinoma were: 76%, 95%, 87%, 91% , and 1p3 mutation for other periampullary carcinomas were 56%, 71%, 82% and 40%, respectively. Duodenal adenocarcinoma had fewer mutations than other carcinomas in the periampullary region, which was not statistically significant. In the entire group, there was a significant correlation between absence of 17q and tumor size and pT ($r=0.4$ and 0.30, $P< 0.01$, respectively), while 3p mutation showed a significant correlation with pT ($r=0.34$, $P< 0.05$).

Conclusions: In this study, molecular markers such as K-ras and 1p3 are helpful in identifying the origin of the adenocarcinoma in periampullary region especially when a large mass is present in the periampullary region, precluding exact idenification of the specific primary origin. Molecular studies can help make a more specific diagnosis with prognostic implications since pancreatic carcinomas have a worse prognosis than the other adenocarcinomas involving this site.

581 Increased Stromal Expression of the Lymphatic Marker D2-40 in the Esophageal Lamina Propria Correlates with Neoplastic Progression to Esophageal Adenocarcinoma

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Background: The monoclonal antibody D2-40, which reacts with the oncofetal membrane antigen M2A, is a novel marker of lymphatic endothelium. While this marker is useful in identifying lymphatic vessels, its expression is now known to be more widespread and occurs in other cell types apart from endothelium. The extent and pattern of D2-40 expression in lymphatic vessels as well as its expression in normal and neoplastic esophagus are unknown. The goal of our study was to determine the pattern of D2-40 expression, degree of lymphangiogenesis, and the extent of lymphatic invasion in the esophageal metaplastic-dysplastic-adenocarcinoma sequence.

Design: Cases of esophageal adenocarcinoma (n=42; 20 with intramucosal invasion and 22 with submucosal invasion), high grade dysplasia (n=12), intestinal metaplasia (n=12), and normal esophagus (n=14) and stomach (n=17) derived from surgically and endoscopically resected esophageal specimens from the Hospital of the University of PA were stained for D2-40 using standard immunohistochemical techniques. Each case was scored (0-3) for lymphatic vessel density and stromal expression of D2-40 within the lamina propria, muscularis mucosa, and submucosal compartments. Lymphatic invasion was defined as tumor within a D2-40 stained space. Statistical analysis was performed using the Mann-Whitney test.

Results: D2-40 expression in stromal cells within the lamina propria in the area of adenocarcinoma (mean score 2.17, median 2) was significantly greater ($p\leq0.0014$) than expression in the lamina propria of high grade dysplasia (1.17, 1), intestinal metaplasia (1.0, 1), stomach (0.82, 1), and squamous esophagus (0.71, 1). In 9/42 (21%) adenocarcinomas, there was rimming of the tumor by intensely D2-40-stained stromal cells. The lymphatic density was not significantly different in any compartment among these categories. Only 1/42 (2.4%) adenocarcinomas had definable lymphatic invasion.

Conclusions: Although lymphatic density did not change along the metaplastic-dysplastic-adenocarcinoma sequence, stromal expression of D2-40 in the lamina propria increased with progression to adenocarcinoma. Thus, stromal D2-40 expression is an early event in esophageal adenocarcinoma progression. This finding further highlights the potential biologic importance of epithelial-stromal interactions in neoplastic progression.

582 The Incidence and Seasonal Variance of Microscopic Colitis in Iceland from 1995-2005

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Background: Microscopic colitis is associated with chronic watery diarrhea and macroscopically normal colons. One study, conducted in Vermont, demonstrated a seasonal variance, raising suspicion that an infectious agent could be responsible. The present study aimed to determine whether a similar seasonal variance could be detected in Iceland, where the reported incidence is the highest in the world.

Design: All cases of microscopic colitis diagnosed at the University of Iceland from 1995-2006 were identified from a computerized search of pathology reports. A slide review was performed on all cases to confirm the biopsy diagnoses. 20 or more lymphocytes per 100 superficial epithelial cells was required to confirm lymphocytic colitis, and a subepithelial collagenous band greater than 14 micrometers thick was required to confirm collagenous colitis. A chart review was performed to document a negative endoscopic examination, exclude clinical features of celiac sprue, and identify the month in which patients first presented with diarrhea.

Results: 321 patients met histopathologic diagnostic criteria. Of these, chart review yielded specific answers to all three clinical questions in 117 cases, which form the final study population. The mean annual incidence was 6.5/100,000 for collagenous colitis, and 5.8/100,000 for lymphocytic colitis. These figures represent an increased incidence since it was previously studied in 2002, and the highest incidence yet reported. Over the ten-year study period, 41 cases occurred during winter months, 21 cases during spring months, 21 cases during summer months, and 34 cases during fall months. The combined fall/winter incidence is nearly twice that of the combined spring/summer incidence. There was a two-fold rise in incidence of lymphocytic colitis between 1999 and 2003.
Conclusions: Iceland has seen an increase in microscopic colitis during the study period, which cannot be explained by increased recognition of the disease alone. The pattern of seasonal variability differed from that described in Vermont, with increased cases in the fall and winter months. This different seasonal prevalence may be explained by the distinctly different Icelandic climate and lifestyle. An exogenous factor may also explain a striking increased annual incidence pattern in lymphocytic colitis, since both the Icelandic population and the Icelandic pathologists remained homogeneous and stable. Our findings lend support to a potentially infectious etiology in at least lymphocytic colitis.

583 Spectrum of Histologic Changes in Colonic Biopsies in Patients Treated with Mycophenolate Mofetil

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Background: Mycophenolate mofetil (MMF), an immunosuppressive agent, is frequently used following bone marrow, renal, cardiac and hepatic transplantation. Diarrhea is a commonly seen side effect which may lead to colonic biopsy in some cases. Histologic changes seen in these biopsies have been reported to mimic self-limited colitis, graft-versus-host disease (GVHD) or inflammatory bowel disease (IBD) in isolated case reports and pose management difficulties. The goal of this study is to define the spectrum of histologic changes in colonic biopsies associated with MMF usage.
Design: All solid organ transplant patients evaluated for diarrhea with colonic biopsy from 1999, when MMF administration was begun, were included in the study. Patients who did not receive MMF were used for comparison. Various histologic features including architectural distortion, apoptosis, inflammatory infiltrate, mucosal erosion/ulceration, Paneth cell metaplasia and mucin depletion were evaluated and scored (scale: 0-2). Clinical history, including immunosuppresants and follow-up, was obtained in all cases.
Results: Twenty-six solid organ transplant patients underwent colonic biopsy for refractory diarrhea during the study period (15 kidney, 4 kidney/pancreas, 4 heart, 2 heart/kidney, 1 liver). 18 were taking MMF at the time of biopsy. Biosies in 16 (89%) cases showed histologic changes. Apoptosis and architectural distortion were seen more frequently in patients receiving MMF as compared to the control group. 2 cases showed marked increase in apoptosis at the crypt bases, very closely resembling colonic GVHD. 2 cases showed features mimicking IBD with architectural distortion. However, lamina propria inflammatory infiltrate was milder when compared to typical IBD cases. None of the cases in our study showed patterns of injury mimicking ischemia or self-limited colitis.
Conclusions: MMF is a commonly used immunosuppressant in organ transplantation, including bone marrow transplantation (BMT). This data shows the histologic changes associated with MMF which can resemble GVHD and IBD. Recognition of this association is important given that GVHD is an important differential diagnosis in BMT cases. Similarly, in some cases, MMF induced changes could lead to erroneous diagnosis of IBD. Increased awareness of the histologic spectrum of MMF induced changes is required by the pathologist to avoid diagnostic errors.

584 Serrated Epithelial Proliferations of the Appendix

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Background: A serrated neoplasia pathway for colorectal tumorigenesis has been proposed for sessile epithelial proliferations that include hyperplastic polyp (HP), sessile serrated adenoma (SSA), serrated adenoma (SA), and mixed lesion (ML). Some of these lesions are associated with microsatellite instability (MSI), and are a proposed precursor of colorectal carcinomas. Serrated lesions in the appendix are often difficult to classify, in particular, diffuse mucosal hyperplasia (DMH) is viewed by some as the appendiceal counterpart of SSA. Vigorous morphologic and molecular studies are lacking, and whether the serrated pathway is operational in the appendix is unclear.
Design: H&E slides from cases diagnosed with appendiceal serrated epithelial lesions were classified according to previously established criteria for colorectal serrated lesions as HP, DMH, SSA, SA, low-grade appendiceal mucinous neoplasm with serrated gland architecture (LAMN), or ML. HP was focal, and DMH was segmental or circumferential. SSA required branching, horizontal and dilated crypts, and small foci of pseudostratification, among other features. Requisites for SA include complex serration pattern, intermediate atypicality, and hypereosinophilic epithelium. Mucinous villous lesions were classified as LAMN even if some gland serration was present. Mixed lesions classified by the highest-grade lesion. Representative cases from each category were selected for molecular analysis. After microdissection, genomic DNA was extracted and PCR was performed for five MSI consensus microsatellite loci.
Results: A total of 44 cases were included, with an age range of 22-90 years (mean= 57.8), and a M:F ratio of 0.7. The lesions were classified as HP (n=2), DMH (n=20), SSA (n=8), SA (n=1), LAMN (n=12), and mixed SSA/LAMN (n=1). Molecular analysis showed a high level of MSI (MSI-H) in one case of DMH with adenocarcinoma of right side of the colon, and 3 cases of low level MSI (MSI-L) in 3 SSAs, 2 with ADC of right side of the colon. Of 8 microsatellite stable (MSS) cases, 3 were DMH, 2 SSA, 2 LAMN, 1 SA, and none with a history of adenocarcinoma of the colon.
Conclusions: Many serrated epithelial proliferations of the appendix fall into the category of DMH or LAMN, and true SAs are uncommon. The MSI data suggest a mixture of MSI and non-MSI pathways in serrated epithelial proliferations of the appendix ("SEPA") exists. Presence of a MSI-H status in a case with an adjacent right-sided colonic adenocarcinoma, as well as MSI-L status in similar cases might suggest a field effect.

585 Vascular and Lymphatic Properties of the Superficial and Deep Lamina Propria in Barrett's Esophagus

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Background: A well-known mesenchymal/epithelial interaction occurs in Barrett's esophagus (BE) characterized by the formation of an acquired, more superficially situated, muscularis mucosa (MM) located between the original (deep) MM and the metaplastic columnar mucosa. The presence of a "double" MM in BE results in the formation of a superficial and deep lamina propria (LP). However, the vascular and lymphatic properties of these distinct anatomic regions are unknown, and, thus, the correct classification, and potential for metastases, of carcinomas that infiltrate these two areas are unclear. The aim of this study was to evaluate the density of blood vessels and lymphatic spaces in the superficial and deep LP, and original submucosa, in patients with BE, and to compare the results with the normal LP and submucosa of squamous lined esophagus, as controls.
Design: Routinely processed tissue sections from esophageal resection specimens of 25 patients with BE, and 12 without BE (lined by squamous mucosa), were immunohistochemically stained with CD31 and D2-40 antibodies and evaluated, by light microscopy, for the number and density (per mm^2) of small (<0.15 mm) and large sized >0.15 mm) blood vessels and lymphatics in both the superficial and deep LP and submucosa of BE patients and the normal LP and submucosa of controls.
Results: The density of blood vessels (44.0 blood vessels/mm^2) and lymphatics (10.2 lymphatics/mm^2) in the superficial LP was similar to the deep LP (43.0 and 12.7, respectively; $p>0.05$) in patients with BE. However, both the density of large blood vessels and lymphatics were significantly greater ($p<0.01$) in the deep compared to the superficial LP. Although the overall *number* of blood vessels and lymphatics was similar in the combined superficial and deep LP in BE compared to control LP, the *density* of blood vessels (70.0/mm^2) and lymphatics (27.7/mm^2) in the control LP was nearly double that of either the superficial or deep LP alone in the BE cases ($p<0.001$). The density of blood vessels and lymphatics in the submucosa of BE was similar to the submucosa of controls ($p>0.05$).
Conclusions: BE patients reveal a significant decrease in blood vessel and lymphatic density in the LP, possibly as a result of increased stromal tissue and/or edema. The newly acquired MM, and its effect on separating the original LP into two (superficial and deep) compartments, probably should not change the manner in which pathologists define submucosal invasion in BE.

586 The Prognostic Significance of Apoptotic Protease Activating Factor-1 Expression in Colorectal Adenocarcinoma

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Background: Apoptosis protease-activating factor-1 (APAF-1), a 130-kD protein, is a key factor in the mitochondrial apoptotic pathway. The objective of this study was to evaluate expression of APAF-1 in colorectal tissues corresponding to multistep carcinogenesis model and assess the value of APAF-1 as a prognostic marker in colorectal adenocarcinomas.
Design: Immunohistochemistry for APAF-1 was performed on the tissue microarray of 38 normal mucosa, 46 adenomatous polyps, 529 colorectal adenocarcinomas and 76 metastatic tumors (lymph node or distant metastasis). Medical records were reviewed and clinicopathologic analysis was performed.
Results: Normal colonic mucosa and adenomas were positive for APAF-1 with no exception (100%). However, in colorectal adenocarcinomas, 119 of 529 cases (22.5%) were positive and 410 cases (77.5%) were negative. Moreover, 67 of 76 metastatic cases (88.2 %) were negative and only 9 cases (11.8%) were positive for APAF-1 expression. These results suggest that loss of APAF-1 expression is relatively frequent late event and plays a role in colorectal tumorigenesis. On the clinicopathologic analyses, correlations between APAF-1 and clinicopathologic factors were seen in tumor location ($p<0.001$), tumor invasion ($p<0.001$), lymph node metastasis ($p=0.021$), AJCC stage ($p=0.02$), Dukes' stage ($p=0.001$) and tumor differentiation ($p<0.001$). The survival analysis was significance in patient age, histologic grade, AJCC stage, lymphatic invasion and vascular invasion ($p<0.001$) but not significance in APAF-1 expression ($p=0.478$).
Conclusions: Loss of APAF-1 expression may play a role in tumorigenesis and tumor progression of colorectal adenocarcinoma.

587 Immunohistochemical Staining for CDX-2, PDX-1, NESP-55, and TTF-1 Can Help Distinguish Gastrointestinal Carcinoid Tumors from Pancreatic Endocrine and Pulmonary Carcinoid Tumors

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Background: Well differentiated neuroendocrine tumors (WDNET) of the gastrointestinal (GI) tract, pancreas, and lung are histologically similar. Thus, predicting the site of origin of a metastasis is not possible on morphologic grounds. Prior immunohistochemical studies of WDNET have yielded conflicting results, and the transcription factor PDX-1 (pancreatic and duodenal homeobox factor 1) has not previously been evaluated in this context. The purpose of this study was to analyze the expression of CDX-2, PDX-1, TTF-1, and NESP-55 (neuroendocrine secretory protein-55), a member of the chromogranin family selectively expressed in pancreatic islets and the adrenal medulla, in primary and metastatic WDNET.

Design: 64 GI carcinoid tumors (5 stomach; 5 duodenum; 31 ileum, including 21 primary and 10 metastatic; 11 appendix; and 12 rectum); 39 pancreatic endocrine tumors (PET) (31 primary, 8 metastatic); and 20 pulmonary carcinoid tumors were immunostained for CDX-2, PDX-1, NESP-55, and TTF-1. The extent of immunoreactivity in tumor cells was recorded.

Results:

SITE (N)	CDX-2	PDX-1	NESP-55	TTF-1
STOMACH (5)	0 (0%)	3 (60%)	0 (0%)	0 (0%)
DUODENUM (5)	0 (0%)	4 (80%)	0 (0%)	0 (0%)
ILEUM (31)	30 (97%)	0 (0%)	0 (0%)	0 (0%)
APPENDIX (11)	11 (100%)	6 (55%)	0 (0%)	0 (0%)
RECTUM (12)	0 (0%)	2 (17%)	1 (8%)	0 (0%)
PANCREAS (39)	7 (18%)	11 (28%)	16 (41%)	0 (0%)
LUNG (20)	0 (0%)	0 (0%)	1 (5%)	7 (35%)

CDX-2 has a sensitivity of 98% and a specificity of 91% for midgut carcinoid tumors. All appendiceal and 81% of ileal carcinoid tumors showed staining for CDX-2 in >75% of cells. The specificity of NESP-55 for PET is 98% and TTF-1 for pulmonary carcinoid tumors is 100%. The panel CDX-2, PDX-1, and NESP-55 has a sensitivity of 64% for PET and 78% for GI carcinoid tumors.

Conclusions: CDX-2 is highly specific for midgut carcinoid tumors, although a subset of PET is also positive. NESP-55 and TTF-1 show high specificity but relatively low sensitivity for PET and pulmonary carcinoid tumors, respectively. PDX-1 is not expressed in ileal or pulmonary carcinoid tumors; thus, positive staining may support origin from the pancreas or other GI sites. A panel of these 4 markers can help predict the primary site of metastatic WDNET.

588 5HT4 Receptor-Immunoreactivity (5HT4-IR) Is Expressed by Non-Neuronal Cells, Including Mast Cells, in Human, Rat and Mouse Gastrointestinal Tracts

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Background: 5HT4 agonists are pro-kinetic and used to treat patients with constipation and constipation-predominant IBS. Although studies describe the pharmacological actions of 5HT4 agonists on human GI samples, there is little data on the morphological localization of 5HT4 receptors in the gut.

Design: Samples of surgically derived tissue from the GI tract and mucosal biopsies from patients with various disorders were obtained with consent. These tissues and rat and mouse colons were formalin fixed. Immunohistochemistry was performed using polyclonal antisera specific for the 3rd cytoplasmic domain (LS655, LifeSpan, WA) and the C-terminus of the common sequence of the splice variants (NLS656, Novus, CO). *In situ* hybridization and double-stains of 5-HT4 with glial fibrillary acid protein, smooth muscle actin, GAP-43 and tryptase were done. Controls included 5HT4b-transfected CHO cells.

Results: Both antibodies labelled a small proportion of transfected CHO cells; however, NLS656 also stained wild type CHO cells, indicating a lack of specificity. 5HT4-IR was localized in the muscularis mucosa, muscularis propria and vessels by both antibodies in all human tissue samples. By in situ hybridization, muscle was also labeled. Significant neuronal staining was not identified; however, with NLS656 there appeared to be perineuronal staining in ganglia, consistent with the labeling of glial cells. Mast cells in all human, rat and mouse tissues were strongly LS655-IR positive and exhibited weak labeling with NLS656. Although not convincingly labeled in the resection samples, in mucosal biopsies enteroendocrine (EE) cells appeared to be 5HT4-IR.

Conclusions: The distribution of 5HT4 was consistent throughout the human GI tract, although the cellular localization varied depending upon the primary antiserum employed. The most consistent 5HT4-IR staining was on muscle cells, while neurons appeared to be negative. The labeling of mast cells in human, rat and mouse tissues, as well as EE cells, suggests that in addition to a direct action on muscle, 5HT4 ligands might act indirectly through these non-neuronal cell types. Results with NLS656 must be interpreted with caution because of the apparent lack of specificity.

589 Nucleolar Expression of Catalytic Subunit of Human Telomerase Reverse Transcriptase (h-TERT) Is Associated with Better Survival in Patients with Stage III Colon Cancer

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Background: Telomerase is a ribonucleoprotein that synthesizes telomeres and is expressed in many malignant cells. The nucleolar expression of the catalytic protein subunit of telomerase, human telomerase reverse transcriptase (h-TERT), has been associated with prognosis in many tumors including those from colon and lung. The correlation between expression of h-TERT in Stage II and III colorectal carcinoma and survival was investigated.

Design: Tissue microarrays of formalin-fixed, paraffin-embedded archival tissue constructed from 97 colorectal resection specimens (45 stage II and 52 stage III) were stained with mouse anti-h-TERT monoclonal antibody (Clone 44F12, dilution of 1:25, Novacastra, Novwel, MA). Each tumor was sampled four times in the array with two cores from the superficial tumor and two from the deep tumor. The percentage of cells with nucleolar staining was scored by light microscopy by a single observer. The mean value from the four samples of each tumor was correlated with survival using the Kaplan-Meier log-rank test performed by SPSS software (version 11.5.2.1 for Windows; SPSS, Chicago, IL).

Results: h-TERT-high, defined by staining of >10% of tumor cells, was observed in 67 tumors (33 stage II and 34 stage III). The mean overall survival of all patients whose tumors had h-TERT-high was 98.3 ± 40.1 months compared to 75.2 ± 48.1 months in patients whose tumors had h-TERT-low (log-rank test, p=0.008). The mean overall survival of stage III patients whose tumors had h-TERT-high was 93.9 ± 45.7 months compared to 55.7 ± 43.9 months in patients whose tumors had h-TERT-low (log-rank test, p=0.0007). In contrast, the mean overall survival of stage II patients with or without h-TERT-high was not significantly different (102.8 ± 33.4 months in patients whose tumors had h-TERT-high and 104.5 ± 39.6 months in patients whose tumors had h-TERT-low).

Conclusions: Nucleolar expression of h-TERT correlates with better prognosis in stage III colorectal carcinoma but not in stage II tumors. The data suggest that differences in prognosis with h-TERT expression are stage-specific.

590 Serrated Adenomas of the Large Intestine: Loss of Crypt Anchoring Is a Defining Feature of Traditional Versus Sessile Serrated Adenomas

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Background: The morphologic distinction between sessile serrated adenoma (SSA) and hyperplastic polyps (HP) may be challenging, but the distinction between SSA and traditional serrated adenoma (TSA) is even more difficult using currently available criteria, which are mostly based on cytological characteristics of the lesions. This study evaluated architectural, cytological and immunohistochemical features of SSA and TSA and provides new and simplified morphologic criteria which can be applied to differentiate between these two types of serrated adenomas.

Design: Thirty SSA, 10 HP, and 10 TSA were evaluated morphologically and immunohistochemicaly. Features evaluated included overall shape of the polyps, architectural features of individual crypts, cytologic dysplasia and other cytologic features of the cells, size and distribution of the proliferation and maturation zones. Expression of Ki67, CD138, and CK20 were also evaluated.

Results: The presence of the stalk was found only in 4/10 TSA and 0/30 SSA and 0/10 HP. Two major architectural abnormalities/events were detected in serrated polyps: abnormal distribution of proliferation zone (as detected morphologically and by Ki-67 expression) and loss of crypt anchoring (LCA). LCA, defined by the presence of apparent malformed crypts with their bases not seated adjacent to the muscularis mucosae, appears to be nearly exclusive to TSA and was found in all cases, while the presence of cytologic atypia and eosinophilia of the cytoplasm were not limited to TSA. LCA was found in small foci of SSA in 2/30 cases and in 0/10 HP. The extent of the expression of CD138, CK20, and Ki-67 could not distinguish between the three types of serrated polyps, but the distribution of their expression confirmed the presence of the LCA in TSA and the absence of the LCA in SSA and HP.

Conclusions: The presence of the LCA defines TSA in a more rigorous fashion than previous diagnostic criteria and also explains the biological basis of "polypoid growth" associated with TSA and the lack of such growth in SSA. Recognition of this phenomenon may also help in exploring the genetic and molecular basis for differences between SSA and TSA, since these architectural abnormalities may well be a reflection of abnormalities in genetically programmed mucosal development.

591 Massive Gastric Juvenile Polyposis: A Clinicopathologic Study

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Background: Juvenile polyposis syndrome, while the most common of the hamartomatous syndromes, is an uncommon condition characterized by the occurrence of multiple juvenile polyps in the gastrointestinal tract. Between 20 and 50% of cases are familial (autosomal dominant with variable penetrance). While the condition usually affects the colon, and less commonly the stomach with small bowel, rare cases limited to the stomach have been reported. Herein we describe the clinical and pathological features of four patients we recently diagnosed with massive gastric hyperplastic/juvenile polyposis.

Design: Routinely processed tissue sections from gastric resections were evaluated for the morphologic features of the multiple gastric polyps encountered. Correlation was made with prior biopsy results and with the medical record to determine clinical features including past medical history, family history (FH) of gastric or intestinal polyps, and laboratory results, including anemia or hypoalbuminemia.

Results: There were 2 men and 2 women (ages 25 to 56) with juvenile polyposis of the stomach, with gastric polyps ranging in number from 40 to several hundred and in size from 0.3 to 6 cm. The polyps were comprised of edematous stroma and a glandular proliferation that largely recapitulated surface and foveolar epithelium without significant atypia. However, one patient had poorly differentiated adenocarcinoma of the stomach, with areas of diffuse type signet ring cell and intestinal type carcinoma, along with innumerable hamartomatous/juvenile polyps with associated intraepithelial neoplasia ranging from low-grade to intramucosal carcinoma. This patient also had juvenile polyps of the small bowel. Another patient had a history of a previous partial gastrectomy with a well-differentiated intramucosal adenocarcinoma arising in a juvenile polyp with high-grade dysplasia. All four patients had a history of anemia (Hct as low as 19%) and one had hypoalbuminemia. One patient had a colon adenocarcinoma arising in a 5 cm tubulovillous adenoma, and two had undergone prior colon resections for juvenile polyposis. One patient had a FH (father) of gastric polyps with features of juvenile polyposis. Only the patient with the poorly differentiated gastric carcinoma is known to have expired.

Conclusions: Massive gastric juvenile polyposis is a rare yet important entity, in that FH and lower GI manifestations are strongly implicated. It warrants close clinical follow-up as severe bleeding, dysplastic change, and even adenocarcinoma can occur within the gastric lesions.

592 Extracellular Sulfatases Are Expressed in Pancreatic Ductal Adenocarcinoma and Regulate Wnt Signaling and Proliferation

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Background: Signaling via the Wnt pathway is critical in embryonic development and remains functional in a limited number of cells within the adult. Aberrant Wnt signaling has been established to be critical in many cancers and has previously been implicated in pancreatic adenocarcinoma associated with increased total and nuclear accumulation of β-catenin. Extracellular heparan sulfate proteoglycans (HSPGs) control Wnt signaling through interactions with Wnt ligands. We have recently cloned and characterized two extracellular endosulfatases, HSulf-1 and HSulf-2. These enzymes remove 6-O-sulfate from HSPGs and modulate their interactions with Wnt ligands, thus initiating Wnt signaling. We hypothesized that HSulf-1 and HSulf-2 are upregulated in human pancreatic cancer, enhance Wnt signaling and promote tumor growth.

Design: Seven archived cases of invasive pancreatic ductal adenocarcinoma were stained with antibodies specific for HSulf-1 and HSulf-2 and staining intensity was assessed. Twenty-four pancreatic cancer cell lines were evaluated by RT-PCR for HSulf expression. The effect of HSulfs on cell proliferation was evaluated by transfection of catalytically inactive HSulfs into cell lines with intact Wnt signaling pathways. Finally, the effect of HSulf-2 silencing was evaluated in an in vivo tumorigenesis assay.

Results: The majority of benign ducts showed no or trace staining for HSulf-1 or HSulf-2, but focal moderate-strong staining for HSulf-1 was seen in the minority (<50%) of benign ducts. 7/7 cases of invasive carcinoma showed moderate to strong staining for HSulf-1 (100% of cases). 4/7 cases (57%) of invasive carcinoma had strong staining for HSulf-2, 2/7 cases (29%) had moderate staining and 1 case (14%) was negative. Staining for HSulfs was not significantly associated with tumor differentiation in this preliminary study. 12/24 pancreatic cancer cell lines expressed HSULF-1 transcripts and 21/24 cell lines expressed HSULF-2 by RT-PCR. Exposing pancreatic cancer cell lines to catalytically inactivated HSulf-2 protein resulted in inhibition of cell growth and Wnt signaling. Silencing of HSulf-2 in a pancreatic cancer cell line also led to attenuated in vivo tumorigenesis.

Conclusions: Our results show that HSulf-1 and HSulf-2 are broadly expressed in pancreatic ductal adenocarcinoma. Taken together with our vitro findings, these results implicate HSulfs in the activation of Wnt signaling and promotion of pancreatic tumor growth.

593 Correlation between p16 Expression, Loss of Heterozygosity and Survival in Advanced Gastric Cancer

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Background: The alterations of tumor suppressor gene p16 (INK4A) have been associated with poor prognosis in many types of cancers. The product of p16 acts as a negative cell cycle regulator by inhibiting G1 cyclin-dependant kinases that phosphorylate the retinoblastoma protein. We have examined loss of heterozygosity (LOH) of p16 on chromosome 9p21 in advanced gastric cancer, and investigated the relationship between allelic loss, p16 protein expression, and cancer survival.

Design: 260 patients with advanced gastric cancers entered into a Southwest Oncology Group (SWOG S9008) protocol were studied. None of the patients received chemo or radiation therapy prior to surgery, and all were randomized to either observation or post-surgical chemoradiation. Tissue microarrays (TMAs) were developed for p16 immunohistochemical analysis. Neoplastic and matched normal tissues from each patient were microdissected from H&E stained slides. DNA was isolated from the microdissected tissues. Microsatellite marker D9S171 for p16 was amplified by PCR and LOH analyzed by ABI310 automatic sequencer.

Results: 13.6% (30/220) p16 LOH cases were identified among 220 informative cases. On immunohistochemical analysis, 40.7 % (24/59) of examined cases lost p16 expression. Among the 24 which were negative for p16 expression, 8.3% (2/24) were positive for LOH; and the 35 case with positive for p16 expression, 14% (5/35) contained LOH. These differences were not statistically significant (p=0.48). For the survival, in the observation arm, the median survival for LOH and normal cases was 38 and 27 months, while in the treated arm the medians were 20 and 38 months respectively. A test of interaction between p16 LOH and patient treatment was significant (p=0.04) suggesting that the patterns of survival between p16 LOH differed by treatment group. Patients with p16 LOH showed significant worse on treatment than those without (p=0.02). Among the p16 expression negative patients, the median survival is 29 months, and it is 30 months in those with p16 positive expression (p=0.83). Thus, there is no evidence to suggest that p16 expression is prognostic for survival.

Conclusions: This study suggests that p16 LOH, but not p16 expression, may serve as a potential marker in the evaluation of patient response to therapy. There is no statistically significant correlation between p16 expression and LOH in advanced gastric cancers.

594 Cytokeratin 7 and CDX2 Immunolabeling Reliably Distinguishes Serrated Polyps of the Colorectum with and without Neoplastic Potential

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Background: In recent years, a subset of colorectal polyps with serrated morphology and abnormal proliferation were recognized, termed sessile serrated adenoma (SSA) to acknowledge their neoplastic potential. However, identification of these lesions is often difficult and ancillary studies to aid recognition are lacking.

Design: We obtained paraffin-embedded samples of 41 colorectal polyps with serrated epithelium from the surgical pathology archives. All samples were reviewed and classified by the criteria of Torlakovic et al (AJSP 2003) into serrated lesions with and without malignant potential for correlation with clinicopathologic features of each patient. Immunohistochemical labeling for cytokeratins 7 and 20 and CDX2 were performed using standard methods, and scored for the presence and absence of labeling, the distribution of positive labeling or the percent of positive labeling in each polyp. Frequencies were compared using the Chi-squared test, or for sample sizes <5 the Fisher exact test. p-values ≤0.05 were considered significant.

Results: 19 hyperplastic polyps (HP) and 22 SSAs from 39 patients were collected. There was no significant difference in patient age (58.9±10.6 yrs vs 63.7±13.1 yrs), race (10W:5B:3n/a vs 15W:2B:4n/a), or gender (8M:10F vs 13M:8F) among each group of patients. Patients with SSA were more likely to have had a prior or coexistent tubular adenoma (13/21 vs 3/18, p=0.004) and more SSAs were located in the right colon than hyperplastic polyps (19/22 vs 3/19, p=0.000006). Immunohistochemical labeling indicated a significant difference in the presence of CK7 labeling among HP and SSA (16/19 HPP versus 6/22 SSA, p=0.004), whereas no difference in CK20 labeling was found. No difference in the sole presence or absence of CDX2 was noted either, although the percent CDX2 labeling was significantly lower in SSAs than in HP (30.0±16.5% vs 52.5±29.7%, p=0.0179).

Conclusions: CK7 ± CDX2 immunolabeling patterns in serrated polyps of the colorectum may have diagnostic utility and improve recognition of serrated polyps with neoplastic potential.

595 Molecular Features of Serrated Epithelial Lesions of the Appendix

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Background: Recent recognition of a serrated pathway of colonic neoplasia has led to the discovery of a previously unrecognized neoplasm, commonly termed "sessile serrated adenoma" (SSA). Serrated lesions may occur in the appendix, but their molecular characteristics are unknown. In this study, we evaluated the pathologic and molecular features of serrated appendiceal lesions in order to gain insight into their pathogenesis.

Design: Routinely processed tissue sections from 34 serrated appendiceal lesions [13 hyperplastic polyps (HP), 10 SSA, 5 serrated adenomas (SA), and 6 mixed SSA/SA)] and 17 controls with non-serrated mucinous cystadenomas (CAD) were evaluated. Immunostains for MLH-1, MSH-2, MSH-6, MGMT, p53, B-catenin, and Ki-67 were performed using the ABC technique and graded for either loss of staining (MLH-1, MSH-2, MSH-6, MGMT) or the presence of nuclear staining (p53, B-catenin, Ki-67). DNA extraction, PCR amplification, and direct sequencing were performed on 12 study cases (3 HP, 5 SSA, 2 SA, 2 mixed SSA/SA) and 4 CAD to evaluate for mutations in BRAF V600E and K-ras codons 12 and 13.

Results: The study patients showed a similar age distribution (mean: 60 vs. 57 years), but a significantly higher male/female ratio (16/18 vs. 2/15, p=0.04) than controls. Loss of MLH1 or MSH-6 occurred in 26 (76%) study cases [7/13 (54%) HP, 10/10 (100%) SSA, 3/5 (60%) SA, 6/6 (100%) SSA/SA], compared to 1 (6%) CAD (p<0.001). Loss of MGMT expression was significantly more common in hypereplastic/serrated lesions (82%) compared to CAD (1/17, 6%, p<0.001), and was present in 8/13 (62%) HP, 10/10 SSA (100%), 4/5 (80%) SA, and 6/6 (100%) SSA/SA. None of the study cases showed nuclear p53 or B-catenin staining, compared to 1 (6%) CAD. Only 2 (6%) study cases (1 SA and 1 SSA/SA) and 6 (35%) CAD showed Ki-67 staining in the surface epithelium. Mutually exclusive BRAF (4) or K-ras (4) mutations were identified in 8/9 (89%) serrated neoplasms [5 SSA (3 BRAF and 2 K-ras), 1 SA (BRAF), and 2 SSA/SA (1 BRAF and 1 K-ras)], but in only 1 CAD (K-ras mutation, p=0.05) and none of the HP cases.

Conclusions: Serrated and non-serrated appendiceal neoplasms have different molecular features. DNA methylation abnormalities involving hMLH-1 and/or MGMT, are seen in most serrated epithelial neoplasms of the appendix, including conventional "HP". Mutations in genes involved in the MAP kinase pathway (BRAF and K-ras) are common in serrated appendiceal neoplasms, similar to their colonic counterparts.

596 Routine Use of Immunohistochemistry for the Mismatch Repair Proteins on Unselected CRC: An Initial Experience

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Background: Immunohistochemical (IHC) stains for the mismatch repair (MMR) proteins help screen for Lynch Syndrome and identify microsatellite unstable colorectal carcinomas (CRC), providing prognostic information. It has been suggested that CRC be screened routinely for a MMR defect, but data are lacking on the practical application of this policy. Our institution began evaluating IHC stains for the MMR proteins in all unselected CRC on March 1, 2006 and we investigated the issues that arose from this new practice.

Design: Surgeons and oncologists were educated regarding the MMR proteins, microsatellite instability and Lynch Syndrome during weekly tumor board conference. Consecutive primary CRC resections regardless of age, family history or histologic features were prospectively stained for the MMR proteins. Stains for MLH1, MSH2, MSH6 and PMS2 were evaluated and scored as absent or present expression. Results were included in the pathology synoptic reports with an explanation of the test and results. Follow-up and reimbursement were evaluated.

Results: A total of 84 CRC were studied including 37 right-sided tumors and 47 left-sided tumors. In one case, all stains were weak and required repeat staining with resultant weak but positive staining possibly due to suboptimal fixation. There was absent expression for 1 or 2 MMR proteins in 9/84 (10.7%) tumors. 6 showed absence of MLH1 and PMS2 expression and patient age ranged from 57 to 91 years old. There were 2 tumors that showed absence of MSH6 expression in patients 42 and 54 years old. One tumor showed absence of both MSH2 and MSH6 in a 53 year old. 5 of the 6 patients with absence of MLH1 and PMS2 were not referred for genetic counseling

because they were over age 80 and as such, the IHC results were likely due to acquired methylation of the *MLH1* promoter. The remaining 4 patients with absent staining have been referred to Cancer Genetics for possible further work-up. The reimbursement rate and turn-around time for the IHC stains were similar to that for other IHC stains used in clinical practice.

Conclusions: IHC stains for the MMR proteins are fast and relatively easy to institute in routine evaluation of CRC, and we have not had difficulty interpreting the stains leading to additional testing. Furthermore, reimbursement was obtained at a level similar to other IHC stains used in clinical practice. The surgeons and oncologists welcomed the prognostic information. Further study is warranted to confirm these initial findings.

597 High Fidelity Image Cytometry in Neoplastic Lesions in Barrett's Esophagus, Including Basal Crypt Dysplasia-Like Atypia with Surface Maturation

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Background: Chromosomal instability and DNA aneuploidy is a key event in the progression of cancer in Barrett's esophagus (BE). Automated image cytometry using high fidelity DNA histograms have been shown to be more sensitive and less susceptible to technical and interpretation issues than flow cytometry for analyzing cellular DNA content. Basal crypt dysplasia-like atypia (BCDA), with surface maturation, has recently been reported to represent an early form of true dysplasia in BE. The aim of this study was to evaluate DNA content in the progression of neoplasia in BE including cases of BCDA, with surface maturation.

Design: Eighty-two formalin fixed mucosal biopsies from 65 BE patients (M/F ratio: 6.3, mean age: 65 yrs.), representing the full range of neoplasia [negative (n = 2), indefinite (n = 2), BCDA (n = 14), low grade dysplasia (LGD, n = 17), high grade dysplasia (HGD, n = 19), and adenocarcinoma (AD, n = 28)] were stained with H and E and Feulgen and evaluated for DNA content using an automated cellular imaging system (ACIS) to generate high fidelity DNA histograms. Histograms consisted of DNA index (DI), representing integrated optical density relative to stromal cells in the same section and were classified into diploid (peak DI = 0.9 – 1.1), mild aneuploidy (peak DI = 1.1 – 1.3), moderate aneuploidy (peak DI = 1.3 – 1.8), and severe aneuploidy (peak DI >1.8).

Results: The prevalence of aneuploidy increased significantly ($p<0.01$) from negative/indefinite (25%) to BCDA (57%), LGD (59%), HGD (100%) and AD (100%). Most aneuploid BCDA (87%) and LGD (100%) were mild in contrast to HGD (32%) and AD (0%), the majority of which showed either moderate (HGD: 26%, AD: 43%) or severe aneuploidy (HGD:42%, AD: 57%, $p<0.01$ vs BCDA or LGD). In addition, both cellular DNA content heterogeneity, and the distribution of cells with DNA content >5N, increased progressively from BCDA and LGD to HGD and AD ($p<0.01$).

Conclusions: High fidelity image cytometry provides objective discriminatory information in BE-associated neoplastic lesions, and may be a useful adjunct to histology and a potential marker of progression of neoplasia in this condition. Ploidy status of BCDA is similar to that of LGD, which supports the concept that this abnormality represents an early neoplastic lesion in BE.

Genitourinary

598 Primary Carcinomas of the Urethra: A Clinicopathologic Analysis of 100 Patients

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Background: Primary urethral carcinomas are rare, accounting for <1% of urinary tract malignancies. Since most of these have been reported as small series or cases reports, their clinical and morphologic spectrum remain incompletely defined, and their behavoir is poorly understood.

Design: Retrospective review of all available material from 100 patients diagnosed with primary urethral carcinoma at one center. Primary urethral carcinomas were defined as tumors arising in the urethra; bladder tumors extending to the urethra were excluded.

Results: Patient data: 29-85 year age range; 61 years median age; 3:2 male:female ratio. Median tumor size of 4 cm; 0.5-6.5cm range. Most common tumor location: proximal urethra in females, bulbar urethra in males. Histological types included 27 urothelial carcinoma (UC), 36 squamous cell carcinoma (SC), 17 adenocarcinoma (AC), 6 mixed carcinomas (MC), 11 carcinoma NOS (CN), 2 sarcomatoid carcinoma and 1 lymphoepithelioma-like carcinoma. AC included: 6 clear cell, 3 mucinous, 3 enteric, 2 signet ring and 3 adenocarcinoma NOS. 7 tumors arose from urethral diverticula, 5 were AC; 6 were in women. Most tumors were grade 3 in UC, moderately differentiated in SC and AC. Pathologic stage at diagnosis: 1 pTa (1 MC); 14 pTis (6 UC, 1 AC, 5 SC, 2 CN); 22 pT1 (6 UC, 3 AC, 8 SC, 4 CN, 1 MC); 18 pT2 (5 UC, 4 AC, 8 SC, 1 MC); 17 pT3 (5 UC, 2 AC, 9 SC, 1 MC); 3 pT4 (1 AC, 1 SC, 1 CN). There were lymph node metastasis in 22 cases (7 UC, 5 AC, 7 SC, 2 CN, 1 MC); right inguinal as the commonest site. Distant metastasis in 15 cases (3 UC, 2 AC, 7 SC, 2 MC, 1 CN): 4 liver, 8 lung, 3 bone, 1 adrenal and 2 pelvic soft tissue. Along with surgery, 42 patients had neoadjuvant therapy (22 chemotherapy, 8 radiation, 12 chemoradiation), and 20 had adjuvant therapy (11 chemotherapy, 3 radiation, 6 chemoradiation). Clinical outcome data: 17 alive with no disease (5 UC, 1 AC, 7 SC, 3 MC, 1 Other); 24 alive with disease (5 UC, 4 AC, 11 SC, 1 MC,3 CN); 28 dead of disease (20.9 months average survival; 11 months median survival; 9 women; 19 men; 7 UC, 4 AC, 9 SC, 2 MC, 4 CN, 2 Other;); 2 dead of other causes (1AC, 1 CN); 25 dead of unknown causes (10 UC, 5 AC, 7 SC, 3 CN). Both sarcomatoid carcinoma patients died of disease (2 and 7 months survival).

Conclusions: Primary urethra carcinomas are tumors of older individuals and are more commonly seen in males. The clinicopathologic features of these tumors are different in men compared to women, with men more likely to die of disease. Sarcomatoid carcinoma has the worst outcome.

599 Anterior Predominant Prostate Tumors: A Contemporary Look at Zone of Origin

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Background: Aggressive PSA screening and prostate needle biopsy protocols have successfully detected low-volume posterior tumors, with a concurrent increase in anterior-predominant prostate cancer (AT). Zone of origin, patterns of spread, and extraprostatic extension of these tumors have not been well studied.

Design: We greatly expanded and refined our previous studies to include pathologic features of 197 patients with largest tumors anterior to the urethra in whole-mounted radical prostatectomy specimens.

Results: Of 197 AT, 97 (49.2%) were predominantly located in the peripheral zone (PZ-D), 70 (35.5%) in the transition zone (TZ-D), 16 (8.1%) were of indeterminate zone (IND), and 14 (7.1%) in both PZ and TZ (PZ+TZ). **PZ-D tumors:** 34/97 (35%) = Gleason score (GS) 6, 61 (62.9%) = GS 7, and 2 = GS 9; 49 (50.5%) involved anterior fibromuscular stroma (AFMS); 83 (85.6%) were organ confined (OC) and 11 (11.3%) had extraprostatic extension (EPE), with invasion of anterior/anterolateral fat; 4 EPE cases also had a positive anterior margin (M+); 63 (64.9%) invaded the apical-most section, 11 with apical M+; 88 (90.7%) showed additional tumors of posterior PZ origin (GS 6: 66, GS 7: 21, GS 8: 1; posterior EPE: 3; posterior M+: 2). **TZ-D tumors:** 24/70 (34.3%) = GS 6 and 46 (65.7%) = GS 7; 52 (74.3%) involved AFMS; 60 (85.7%) were OC and 7 (10%) showed EPE, with invasion of anterior fat (7/7) and bladder neck [BN] (2/7); 3 EPE cases and 3 OC tumors showed anterior M+; 46 (65.9%) invaded the apical-most section, 4 with apical M+; 64 cases (91.4%) showed additional PZ tumors (GS 6: 45, GS 7: 19; posterior EPE: 3; posterior M+: 2 [1 OC, 1 EPE]). **IND tumors:** 5/16 (31.2%) = GS 6 and 11 (68.8%) = GS 7; 13 involved the AFMS without clear relationship to either TZ or PZ; 12 were OC, 4 had EPE with involvement of anterior fat (4/4) and BN (1/4); 15 cases had additional PZ tumors (GS 6: 12; GS 7: 3). **PZ+TZ:** 2/14 = GS 6 and 12 = GS 7; 12 were OC, 1 with anterior M+; 2 showed EPE, one with anterior/apical M+; 10 involved AFMS; 8 involved the apical-most section, 1 with M+; 13 had other PZ tumors (GS 6: 10; GS 7:3).

Conclusions: PZ-D tumors are more prevalent than TZ-D tumors in the anterior prostate. AT of both zones show similar GS, EPE, and invasion of the apical portion of the prostate. TZ-D tumors more commonly involve AFMS. Tumor extension into anterior fat may represent definitive EPE in this region, where no clear capsule is present. Prostates with AT frequently contain additional PZ tumors which are occasionally stage-determining.

600 Prostate Specific Membrane Antigen (PSMA) Expression in Primary Prostatic Adenocarcinoma: Comparison of Expression with Gleason Grade and Hormonal Therapy

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Background: Prostate specific membrane antigen (PSMA) is a type II transmembrane glycoprotein that is consistently expressed in benign and neoplastic prostatic tissue with its strongest and most diffuse expression being in prostatic adenocarcinoma (PCa). Antibodies directed against PSMA are being utilized in imaging studies for patients with advanced PCa and its efficacy as a therapeutic agent is presently being studied in clinical trials. Previous studies have used proprietary antibodies with limited availability. Taking advantage of a recently developed and commercially available antibody, we studied PSMA expression by immunohistochemistry (IHC) in a large number of hormone naïve primary PCa (PCaN) and after neoadjuvant therapy (PCaT).

Design: Slides from tissue microarrays blocks prepared from formalin-fixed, paraffin-embedded archival material from prostatectomies performed at Memorial Sloan-Kettering Cancer Center were stained by IHC with monoclonal antibody directed against the external domain of PSMA Clone 3E6 (Dako, Carpinteria, CA). The study included 141 PCaN and 106 PCaT. Hormone-naïve non-neoplastic prostatic tissue (NPN) from 316 cases and 66 cases of non-neoplastic prostatic tissue following neoadjuvant therapy (NT) were also included. The extent and intensity of stain were evaluated semi-quantitatively.

Results: Overall, PSMA expression was detected in 140 of 141 PCaN (99.3%). Expression increased in extent and intensity from NPN to PCaN and was most intense and diffuse in tumors with high Gleason score ($p \leq 0.05$) (Table.1). In PCaT, PSMA expression was detected in 92 of 106 cases (86.8%). In non-neoplastic prostatic acinar cells, PSMA expression was present in 86.7% and 92.4% of NPN and NT, respectively.

Conclusions: Utilizing this commercially available antibody, the majority of PCa express PSMA. This expression is stronger and more widespread in PCa with higher Gleason score. Even after neoadjuvant therapy, PSMA expression is retained in both PCa and non-neoplastic prostatic tissue. PSMA is a useful diagnostic tool for PCa independent of grade and hormonal status.

Table.1: PSMA expression in hormone-naïve prostatic tissue

	NPN	GS 3+3	GS 3+4	GS ≥4+3
Negative	42	1	0	0
Focal	75	10	4	2
Diffuse	199	39	34	51
Weak	111	7	3	2
Strong	163	42	35	51

GS: Gleason score