Conclusions: *RAP1GAP* is likely to serve as a tumor suppressor gene which is frequently affected by LOH and lose expression in a significant proportion of thyroid cancers, especially in those with invasive growth. The loss of the RAP1GAP protein can be detected by immunohistochemistry and may serve as a diagnostic marker of malignancy in thyroid nodules.

Gastrointestinal

542 Quantitative Analysis of Intramucosal Mast Cells in Inflammatory Bowel Disease (IBD) and Symptomatic Non-IBD Patients with Histologically Uninflamed Colon Biopsies

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Background: Increased intestinal mast cell count, as recently proposed by >20 mast cells per high power field (HPF), has been reported in patients with irritable bowel syndrome (IBS). Mast cell activation has also been implicated in IBD but whether the number of intramucosal mast cells is quantitatively increased remains controversial. We aimed to study the density of mast cells in histologically unremarkable or uninflamed colon biopsies obtained from IBD and non-IBD patients to look for any particular disease group(s) that might show an increased mast cell count.

Design: Random colon mucosal biopsies (n=118) showing no histopathologic abnormality (including quiescent IBD) were immunostained with CD117 (c-kit) and mast cell tryptase (MCT). Intact mast cells with an identifiable nucleus were quantified in three 400X fields in areas with the highest density for each stain. The mean values of each case were analyzed according to patient groups based on clinical history and endoscopic findings.

Results: Both CD117 and MCT stains were equally sensitive in detecting intramucosal mast cells with similar mean counts (P=0.864), and thus only CD117 results were subjected to subsequent analyses. The overall mast cell counts in all biopsies ranged from 2-31 per HPF (mean: 16.9±6.2; median: 17). No significant difference was demonstrated between patients with IBD (mean=15.6± 5.6; n= 47) vs non-IBD (mean=17.8±6.4; n=71; P=0.060), Crohn (mean=14.1±6.0; n=21) vs ulcerative colitis (mean=17.0±5.1; n=25; P=0.084), non-IBD with diarrhea (mean=18.3±6.1; n=33) vs without diarrhea (mean=17.4±6.8; n=38; P=0.550), non-IBD with abdominal pain (mean=17.1±6.0; n=19) vs without abdominal pain (mean=18.1±6.6; n=52; P=0.567). Thirty-six biopsies (30.5%) showed >20 mast cells/HPF, which appeared to be equally distributed among various patient groups (23.4% in IBD, 36.4% in non-IBD with diarrhea, and 34.2% in non-IBD without diarrhea; $P \geq 0.221$).

Conclusions: There is no significant difference in the number of intramucosal mast cells in patients with or without IBD, diarrhea, or other digestive symptoms. Increased mast cell counts were observed in various patient groups with uninflamed colon biopsies, suggesting a nonspecific finding that may not be reliable in segregating patients with IBS.

543 Minichromosome Maintenance Protein 7 and SMAD 4 Expressions in Squamous Cell Carcinoma of the Esophagus

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Background: Minichromosome maintenance protein-7 (MCM–7) plays a critical regulator of DNA replication as a component of the DNA replication licensing complex. Recently it has been shown that MCM 7 protein could be correlated with the clinicopathological profiles of some human tumors. In the present study, we evaluated the expression of MCM7 in squamous cell carcinoma of esophagus to clarify the pathobiological significance including prognostic relevance, correlation with clinicopathological characteristics and with that of one of tumor suppressor gene, SMAD4.

Design: We examined the immunohistochemical expression of MCM7 and SMAD4 in 67 surgically resected esophageal specimens, which consisted of 16 early cancer and 47 late-stage(II-IV) cases. Twenty-seven cases(40.3%) showed lymph node metastasis. The overall 5-year survival rate was 56.7%. Distinct nuclear staining of MCM7 and cytoplasmic staining of SMAD4 was considered as positive. The percentage of tumour cells positive for MCM7 was classified into four groups: 1 (< 5-25%), 2 (25–50%), 3 (51–75%) and 4 (> 75%). For statistical analysis, those with negative and positive cases were compared first. The labeling indices of each four group were also compared.

Results: The positivity of MCM7 was 79.1% and significantly correlated with the T status (P=0.008), N status(P=0.032), UICC stage (P=0.03), survival period(p=0.036) and survival(P=0.047). The positivity of SMAD4 was 26.9%, and was not significantly associated with clinicopathological characteristics. Kaplan-Meier survival curves showed that the patients with positive- MCM2 had a poorer prognosis ($P < 0.05$), however those with positive-SMAD4 had no prognostic significance.

Conclusions: These results indicate the expression of MCM7 may be a useful poor prognostic marker in squamous cell carcinoma of esophagus. However, the clinical implication of SMAD 4 expression could not be demonstrated here.

544 Colorectal Lymph Node Examination: How Extensive Should It Be and Why Is "12" the Magic Number? A VAMC Experience

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Background: Twelve lymph nodes (LN) is a current benchmark in LN retrieval in colorectal resections according to the National Quality Forum. Data shows that more extensive lymphadenectomy improves overall survival (OS). These are multi institutional studies which have many variables such as length of colonic fat resected by many surgeons, and gross examination by many path labs. By using one surgeon's case log and one pathology group, we have the opportunity to reduce these variables. Our aim is to determine the impact of extensive LN search on OS, and the impact of 12 LN as a QA standard.

Design: We reviewed 157 patients from one surgeon's case log between 1994-2007. Within the 12 years, one surgeon applied strict guidelines to remove approximately the same distribution of pericolonic fat and the lab followed a 2 step approach to LN dissection. Step 1 is careful search for grossly identifiable LN, and step 2 is overnight immersion in Carnoy's solution to find smaller LN. We used Statview, Kaplan Meier Analysis and SAS Institute software to analyze the following variables: 1) The total number of LN identified and its impact on AJCC stage and OS 2) The OS and its association with finding of >12 LN 3) The impact of number of positive LN on OS.

Results: Of 157 cases, the mean number of identified LN for all resections was 14.75 1) The total number of LN identified did not correlate with AJCC stage (p=0.42) Mean number of LN per stage: I=13.4 (N=41); II=16.3 (N=56); III=14.3 (N=38); IV 14.2 (N=22). Regardless of stage, total number of LN identified was not statistically significant in predicting OS (p=0.24) 2) Regardless of tumor stage, no statistically significant impact on OS was found if <12 or >12 total LN were identified (p=0.06) 3) Regardless of tumor stage, higher number of positive LN was associated with lower OS (Survival decreases by 0.15 month per each positive node, p= 0.001).

Conclusions: 1) Total number of LN identified does not impact AJCC stage or OS 2) Higher number of positive LN alone is associated with worse OS In our experience, the two step LN isolation is an effective way to exceed the National Quality Forum requirement of 12 LN. However, after following our own guidelines for over ten years, our findings suggest that the second step of using carcinogens like Carnoy's solution may not be needed. Twelve lymph nodes may not be a magic number for QA and should be reconsidered.

545 Ampulla of Vater: Morphologic, Clinical, Survival, and Second Colon Primary Cancers Based on 5,625 Cases from the SEER Program

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Background: Cancers of the ampulla have been infrequently studied, especially at a population level. Herein, we report the epidemiologic and morphological characteristics of cancer of the ampulla of Vater and its relationship with primary carcinomas arising in the colon.

Design: In SEER, all patients with a diagnosis of carcinoma of the ampulla were identified between 1973 and 2005. The demographic features, five-year survival rates according to stage, grade, and histologic tumor type, distribution of histological types, and frequency of a primary colon cancer preceding or following the development of the ampullary cancer were compared.

Results: A total of 5,625 cases of ampullary cancer were identified. Ampullary cancers have increased annually since 1973. In both blacks and whites, the disease is more common in men than in women. Adenocarcinomas, NOS comprised 65% of histologic types. Five-year relative survival depends on stage of disease, grade, and histological tumor type. Papillary carcinomas and carcinomas arising in adenomas had a significantly more favorable survival than other types. 10% of patients with ampullary cancer had a preceding primary cancer in another site. Of 571,304 cases of primary colorectal cancer, 134 developed a second primary in the ampulla. Twelve patients were between 15 and 30 years of age. Of 5,625 patients with primary cancer of the ampulla, 59 had second primary cancers in the colon or rectum.

Conclusions: The histological type, grade, co-existing adenoma, and stage serve as prognostic factors. The location of either first or second primary cancers in the colon or rectum associated with cancers in the ampulla followed the frequency distribution of all primary colon and rectal cancers seen in all patients.

546 Usefulness of p16 Immunohistochemistry in the Diagnosis of Lynch's Syndrome

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Background: MLH1 inactivation may be observed in sporadic and Lynch's syndrome colorectal carcinoma (CRC). Lynch syndrome is caused by germline mutations in the mismatch repair genes. Sporadic CRC is caused by epigenetic silencing of MLH1, because of its promoter methylation. These tumours are due to hypermethylation of multiple genetic locus, one of them, *CDKN2A* (*p16*). The aim of this study is to evaluate the value of p16 immunohistochemistry in the prediction of germline *MLH1* mutation in patients with CRC that show loss of MLH1 expression.

Design: The study was performed in 89 tumours with loss of MLH1 immunohistochemical expression from patients of the Genetic Counselling in Cancer Department of HGUE and from a series of non-selected surgical CRC specimens from the EPICOLON study and the Pathology Department of the HGUA. Immunohistochemical analysis for p16 was performed on tissue microarray. The MLH1 and *CDKN2A* (p16) methylation analysis was performed by Methylight. *BRAF V600E* mutation was detected using specific TaqMan probes by real time PCR. In 54 tumours, mutation analysis of *MLH1* was performed.

Results: Loss of p16 expression was seen in 21 out of 76 valuable samples (27.5%). All tumours with loss of p16 expression showed hypermethylation of p16 (21/21, p<0.001), 95.2% (20/21, p<0.005) showed MLH1 methylation and 66.7% (14/21, p<0.005) were mutated for *BRAF V600E*. Values of diferent strategies for detecting Lynch Syndrome are shown.

Strategies for detecting Lynch syndrome

	Sensivity	Specifity	PPV	NPV	OR
P16 IHC	25	100	100	32	1.5 (1.1-1.8)
BRAF V600E	29	100	100	43	1.8 (1.4-2.3)
MLH1meth	53	100	100	80	4.9 (2.7-8.8)
Bethesda	32	100	100	52	2.1 (1.5-2.9)
P16 IHC + BRAF + Bethesda + MLH1meth	63	100	100	86	7.3 (3.5-15.4)

IHC: immunohistochemistry, meth: methylation S: sensivity, Sp: specifity, PPV: positive predictive value, NPV: negative predictive value, OR: odds ratio

Conclusions: p16 immunohistochemistry is a good surrogate marker for *CDKN2A* (p16) and *MLH1* epigenetic silencing due to hypermethylation, and could be useful as a screening tool in the selection of patients for genetic testing in Lynch syndrome. The use of this technique could avoid germline testing in approximately a third of patients with loss of MLH1 expression.

547 Outcome Analysis of 15-Year Follow-Up in a Regional Veterans Affairs Barrett's Esophagus Cohort with Respect to the Updated 2008 Guidelines for Surveillance

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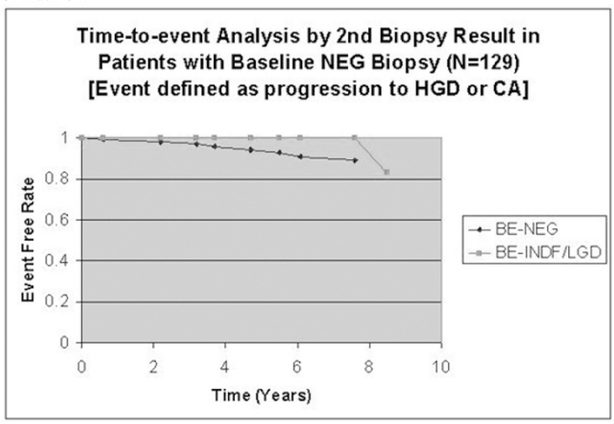
Background: The grade of dysplasia determines surveillance interval in Barrett's esophagus (BE). *American College of Gastroenterology* & *Practice Parameters Committee* published 2008 guidelines: 3-yr surveillance with 2 biopsies (bxs) (within 1 year) negative for dysplasia (NEG); 1-yr surveillance until no dysplasia in 2 bxs when low-grade dysplasia (LGD) is the highest grade on repeat bx (within 6 months). We performed an outcome analysis of our BE cohort to investigate same for our patients (pts).

Design: BE cases between 7/90 and 12/95 were identified. Records were retrospectively reviewed through 6/06 to select cases NEG on 1st bx and with at least 1 follow-up bx. Bxs were categorized as NEG, indefinite (INDF), LGD, high-grade (HGD), or carcinoma (CA). Event was defined as progression to HGD/CA. Time-to-event (TTE) analysis was performed by comparing event free rates between group 1-NEG on 2nd bx and group 2-INDF/LGD on 2nd bx. Kaplan-Meier method with log-rank test was used.

Results: A total of 699 bxs occurred in 131 pts with 859 pt-yr follow-up (1.3 events/100 pt-yrs). 8 of 11 events occurred in group 1 (N=113) with 2 of 8 occurring in <3 years of index bx.



Five of 8 events occurred without INDF/LGD with mean period of 3.4 yrs (R=0.6-7.6) from index bx. The mean time interval between first two NEG bxs was 1.6 yrs (R=0.003-10.04). In 32 of 113 cases, a subsequent diagnosis of INDF/LGD was made with mean of 4 (R=2-13) interval NEG bxs over 5.5 yrs (R=0.5-12.5). In group 2 (N=16), a single event of HGD, identified on the 7th bx, occurred 8.5 yrs from index bx following 2 consecutive NEG bxs.

Table 1: Time-to-event Analysis by 2nd Bx; p=0.942 (N=129)*

	Event Free Rate				
	1-Year	3-year	5-Year	10-Year	15-Year
NEG (N=113)	0.99	0.98	0.94	0.89	0.89
INDF/LGD (N=16)	1.00	1.00	1.00	0.83	0.83

*Two patients with HGD on 2nd bx were excluded.

Conclusions: Time-to-event rate is unpredictable and selected BE-NEG pts may benefit from annual surveillance endoscopy. Continued surveillance may be considered for selected LGD pts despite consecutive NEG bxs.

548 ProEx C Is More Sensitive Than p16INK4a in Detecting HR-HPV in Problematic Squamous Lesions of the Anal Canal

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Background: ProEx C is a potential surrogate marker for high-risk HPV (HR-HPV) in the uterine cervix and anal canal. To date, no study has correlated ProEx C staining with the presence of HR-HPV DNA detected by PCR in clinical samples from either site. We compare ProEx C, p16INK4a and Ki-67 reactivity patterns within various anal squamous lesions and correlate these findings with the results of PCR for HR-HPV DNA in all cases.

Design: We examined 81 anal lesions [14 non-dysplastic (acrochordons, hemorrhoids), 34 low-grade SIL, borderline and atypical lesions (LSIL/AIN I/condyloma; SIL1-2), 17 high-grade SIL (HSIL/AIN II-III/CIS), and 16 invasive squamous carcinomas] from 51 patients [32 M, 19 F; average age 42.7 yrs] to determine the pattern of ProEx C (Tripath Imaging Inc), p16INK4a (CINtec, mtm Laboratories) and Ki-67 (1:200, Dako) reactivity. ProEx C was scored as negative (basal staining only), focal (less than 1/2 of mucosa), or diffuse (greater than 1/2 of mucosa). p16INK4a was scored as negative (blush or strong in less than 5%), focal (strong in 5-80%) or diffuse (strong in greater than 80%). Ki-67 was scored positive when staining the upper 2/3 of mucosa. PCR for HR-HPV DNA was performed in all cases; pyrosequencing was completed on a subset of positive cases.

Results: ProEx C and p16INK4a were equally sensitive in detecting HR-HPV within invasive carcinomas and HSIL. In contrast, p16INK4a failed to stain 12 of 25 HR-HPV (PCR-positive) cases within the LSIL/borderline/atypical group. ProEx C was negative in only 1/25 of these cases. 14/14 non-dysplastic lesions were negative for ProEx C, p16INK4a and Ki-67, and no HPV DNA was detected.

Conclusions: This is the first study to compare ProEx C staining with the presence of HR-HPV DNA detected by PCR in either the uterine cervix or anal canal. Our results indicate that HR-HPV (most commonly HPV16) is present in the majority of high-grade lesions (invasive carcinomas, dysplasias) of the anal canal and a high number of anal condyloma. While both ProEx C and p16INK4a may be useful in the diagnosis of high-grade lesions of the anal canal, ProEx C was markedly more sensitive than p16INK4a in detecting HR-HPV within the problematic low-grade and borderline lesions.

549 Expression of Gastric Mucin MUC6 in Colonic Serrated Polyps

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Background: Serrated lesions of the colon are a heterogeneous category for which consistent application of diagnostic histologic criteria remains challenging. Previous studies have shown variable reproducibility in the distinction of serrated lesions based on molecular and immunohistochemical features. Recently, gastric-type mucin MUC6 expression has been reported to distinguish serrated lesions for improved diagnosis. We compared the expression of MUC6 in proximal and distal serrated polyps.

Design: We identified and described the histologic features and location of serrated lesions obtained from 3792 participants from two large Phase III chemoprevention trials. Immunohistochemistry for MUC6, beta-catenin, p16, hMLH-1, and O-6-methylguanine-DNA methyltransferase (MGMT) were performed on 92 serrated lesions. Distribution of positivity was assessed using percentage expression and a grading scale was used for staining intensity.

Results: Forty-nine hyperplastic polyps (HP), 29 sessile serrated adenomas (SSA), 12 traditional serrated adenomas (TSA), and two mixed lesions (one SSA/HP and one SSA with areas of cytologic dysplasia, SSAD) were included in the study. The mean lesion size was 5mm, ranging from 1-20mm. Seven SSA's (24%), one TSA (8%), and six HPs (12%) were positive for MUC6, but none of the mixed lesions were positive. Immunostaining for MUC6 was limited to the lower crypts in all lesions with the exception of one SSA that had greater staining intensity on the surface. Positive staining of cells in the basal crypts ranged from 5-100% and was independent of histologic type. The difference in MUC6 expression was not significant based on location or size. Beta-catenin expression was primarily cytoplasmic and membranous with no nuclear localization. There was no significant beta-catenin expression differences between the lesions, or glandular versus surface location. Only the SSAD showed loss of hMLH-1. Expression of p16 and MGMT were not significantly different between lesions.

Conclusions: In contrast to previous studies, site-specific or selective expression of MUC6 could not be used to distinguish between serrated adenomas or hyperplastic polyps. No significant differences were found among serrated lesions with regard to loss of hMLH-1, or beta-catenin, p16 or MGMT expression.

550 Identification of Protein Expression Signatures in Gastric Carcinomas Using Clustering Analysis

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Background: The identification of gastric carcinomas (GC) has traditionally been based on histomorphology. Recently, DNA microarrays have been used successfully to identify tumors through clustering of the expression profiles. It has been shown that many tumors can be clustered into clinically relevant groups based solely on gene expression profiles. The expression profiles and molecular grouping of GC has been a challenging task because of their complexity and variation. Random forest clustering is attractive for tissue microarray and other immunohistochemical data since it handles highly skewed tumor marker expressions well and weighs the contribution of each marker according to its relatedness with other tumor markers. In this study we identified biologically and clinically meaningful groups of GC by hierarchical clustering analysis of immunohistochemical protein expression.

Design: We selected 28 proteins (p16, p27, p21, cyclin D1, A, B1, pRb, p53, c-met, c-erbB-2, VEGF, TGFβI, TGFβII, MSH2, bcl-2, bax, bak, bcl-x, APC, clathrin, E-cadherin, β-catenin, MUC1, MUC2, MUC5AC, MUC6, MMP2 e MMP9) to be investigated by immunohistochemistry in 482 GC. The data analyses were done using a random forest clustering method (TMEV-http://www.tm4.org/mev.html). It is an unsupervised learning method, which aims to find molecular classifications with distinct global expression profiles blinded to clinicopathological covariates. We used several statistical methods for describing the clusters in terms of clinicopathological variables and tumor marker expression.

Results: Proteins related to cell cycle, growth factor, cell motility, cell adhesion, apoptosis, and matrix remodeling were highly expressed in GC. We identified proteins expressions associated with poor survival in diffuse type of GC including p53 and TGFβII. Based on analysis of proteins expressions, a two-way clustering algorithm distinguished two groups (clusters) of GC. We also found that clinicopathological covariates differ across clusters (metastases status and TNM stage). In addition, the clustering analysis identified a cluster of diffuse GC associated with better survival.

Conclusions: Our study identified: 1) two groups of GC that could not be explained by any clinicopathological variables, and 2) a subgroup of long - surviving diffuse GC patients with a distinct molecular profile. These results provide not only a new molecular basis for understanding biological properties of GC, but also better prediction of survival than the classical pathological grouping.

551 Only Two Microsatellite Markers (Bat 26 and Bat 34c) Are Needed, with Immunohistochemistry, To Accurately Detect High Microsatelite Instability Cancers

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Background: Microsatellite instability (MSI) is a consequence of dysfunctional mismatch repair proteins MLH1, MSH2, MSH6, or PMS2, which is seen in sporadic colon carcinomas and in cancers associated with the Hereditary Non-Polyposis Colorectal Cancer Syndrome (HNPCC). In addition to immunohistochemistry (IHC) of these proteins, molecular testing for MSI is performed, which consists of PCR and electrophoresis of 10 microsatellites. If >30% of the microsatellites are unstable, the diagnosis is MSI-high, with increased risk for HNPCC, and further genetic testing is performed. Testing 10 markers is costly and time-consuming, so the minimum number of markers that were needed, in conjunction with IHC, for accurate diagnosis of MSI-high, was assessed.

Design: 181 consecutive MSI and IHC results were reviewed. Each of 10 MSI markers (Bat25, Bat 26, Bat 34c, Bat 40, D17S250, D5S346, D18S55, D10S197, MYCL1, ACTC) were independently assessed for association with IHC results and with MSI-high (3+ unstable markers), MSI-low (1-2 unstable markers) or MSS (microsatellite stable; no unstable markers).

Results: 154/181 (83%) of the cancers were colorectal, with the remainder from ovary, endometrium, appendix, omentum, skin, pancreas, and duodenum. 25 cases (8.3%) were MSI-high, and all of these (100%) had loss of expression of at least one IHC marker: 24 negative and 1 weak. 19 cases (10.4%) were MSI-low, of which one had loss of IHC expression. All 137 MSS cases had intact IHC staining. Therefore, the sensitivity of IHC for MSI-high was 100%, with 99.3% specificity. Of the MSI markers, BAT26 and BAT34c alone were sufficient to correctly classify every tumor as either MSI-high or not. All 25 (100%) of MSI-high cases were unstable with at least one of these two markers, and all of the 156 (100%) MSI-low and MSS cases were stable with both of these markers.

Conclusions: In 181 consecutive samples, only two markers, BAT26 and BAT34c, were needed to make this diagnosis with absolute (100%) accuracy. IHC also detected every case of MSI-high, with only one false positive result in a case of MSI-low. While these findings need corroboration from other studies, the implication is that IHC and a more limited MSI panel could be used, reducing time and cost, without impairing diagnostic accuracy. Limiting the MSI panel to BAT26 and BAT34c would have misclassified MSI-low cases as MSS, but this is of no clinical consequence, as MSI-low is not associated with HNPCC and does not require further genetic assessment.

552 The Clinical Significance of Lymph Node Retrieval Using Fat Clearing Techniques in Rectal Cancer Patients Following Neoadjuvant Chemoradiotherapy

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Background: Lymph node status is the single most important prognostic predictor in colorectal carcinoma. It has been shown that lymph node retrieveal is decreased in the setting of preoperative chemoradiation (CRT) Fat clearance techniques have been shown to increase lymph node harvest; however its role in clinical practice remains controversial. The aim of this study was to investigate the application of fat clearance in cases following neoadjuvant chemoradiation.

Design: All patients who underwent proctectomy following neoadjuvant CRT were included. The lymph node numbers were recorded and compared between the traditional method and fat clearance method. Other tumor chracteristics including stage and tumor regression factor were also studied. Results were expressed as mean ± SEM. A $p<0.05$ was considered significant.

Results: From Jan 1998 to Sep 2007, 237 patients were identified, 157 cases in the neoadjuvant therapy group and 80 cases in the non neoadjuvant therapy group. In both groups, patients were divided into traditional lymph node dissection method group (visualization and palpation) and fat clearance method group. In the non neoadjuvant therapy group, there was no significant difference in the number of positive lymph nodes ($P=0.347$) or N+ stage ($P=0.332$) between the two methods, even though the total lymph node harvest was increased significantly by utilizing the fat clearance method ($P<0.001$). In contrast, the total lymph node retrieval ($P<0.001$), number of positive lymph nodes ($P=0.007$) and N+ stage ($P=0.006$) were all increased by fat clearance in the neoadjuvant group. Moreover, the number of cases with N+ stage was compared between the two methods at different T stage level (T0-T4) to eliminate the background bias and the results were confirmed.

Conclusions: In our experience, the utilization of fat clearance significantly influences lymph node staging in rectal cancer following neoadjuvant chemoradiation. These findings suggest that fat clearance may represent a useful tool in all cases receiving neoadjuvant therapy; a more generalized application in colorectal carcinoma specimens remains controversial and warrants further investigation.

553 K-RAS Mutational Analysis in 562 Consecutive Colon Carcinomas

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Background: Oncogenic mutations in K-RAS, the human homolog of the Kirsten rat sarcoma-2 virus oncogene, results in a constitutively active protein in approximately 30% to 50% of colorectal carcinomas (CRC) and has also been described in carcinomas of the pancreas, lung, head and neck and ovary. KRAS encodes a small GTP binding protein that acts as a self-inactivating signal transducer by cycling from GDP- to GTP-bound states in response to stimulation of cell surface receptors, including EGFR. Several recent studies have shown that the presence of a KRAS mutation in CRC correlates with poor prognosis, and is associated with lack of response to EGFR inhibitors. The purpose of this study was to confirm the frequency of mutations reported in the literature as well as to determine the distribution of the specific mutations identified by PCR.

Design: Formalin-fixed, paraffin-embedded tumor sections were deparaffinized and cells of interest were identified and removed using a PALM laser capture microdissection system (Zeiss, Jena, Germany). DNA was isolated from the captured cells using the DNA mini kit (Qiagen, Valencia, CA). Mutant KRAS was detected using a validated KRAS mutation kit (DxS Ltd, Manchester, United Kingdom) that identifies seven somatic mutations located in codons 12 and 13 (Gly12Asp, Gly12Ala, Gly12Val, Gly12Ser, Gly12Arg, Gly12Cys, and Gly13Asp) using allele-specific real-time polymerase chain reaction. Amplification and detection were performed on an Applied Biosytems 7900HT (ABI, Foster City, CA).

Results: Five hundred sixty-two consecutive colon carcinomas (primary and metastatic) were evaluated. Four samples failed to yield a result. Of the 558 analyzed samples, 370 (66%) revealed wild-type K-RAS while 188 (34%) revealed a mutation. The distribution of mutations was as follows Gly12Asp (GGT->GAT) 77 tumors (13%), Gly12Val (GGT->GTT) 51 tumors (9%), Gly13Asp (GGC->GAC) 33 tumors (6%), Gly12Ala (GGT->GCT) 12 tumors (2%), Gly12Cys (GGT->TGT) 9 tumors (1.6%), Gly12Ser (GGT->AGT) 4 tumors (0.7%) and Gly12Arg (GGT->CGT) 2 tumors (0.4%).

Conclusions: The detection rate of K-RAS mutations was consistent with the 30-40% described in the literature. Although all seven mutations known to be associated with lack of response to EGFR inhibitors were identified, five mutations, Gly12Asp (GGT->GAT), Gly12Val (GGT->GTT), Gly13Asp (GGC->GAC), Gly12Ala (GGT->GCT), and Gly12Cys (GGT->TGT), accounted for 97% of the mutations detected.

554 HER2 Status in 146 Gastroesophageal Carcinomas Assessed by Two Rabbit Monoclonal Antibodies (SP3 and 4B5) and Two In Situ Hybridisation Methods (FISH and SISH)

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Background: The incidence of adenocarcinomas of the distal esophagus / gastric cardia is rapidly increasing. Though the number of carcinomas of the gastric body / antrum are decreasing in the industrialized world, these tumors still represent a significant health problem. In recent years, HER2 has emerged as a routinely performed predictive test in breast cancer. Several reports claim significant HER2-positivity in gastro-esophageal adenocarcinomas. At present, a large phase III trial in which HER2-positive gastric cancer patients are treated with trastuzumab – a therapeutic anti-HER2 antibody - is ongoing. New HER2 assessment methods using were introduced recently and are gaining popularity in breast cancer.

Design: HER2 status was examined in gastro-esophageal carcinomas, comparing SP3 (Labvision) and 4B5 (Ventana) immunohistochemistry (IHC), conventional dual probe HER2 FISH (DAKO), and SISH (Ventana) in a Dutch population from a single institution.

Results: IHC was carried out on biopsies of 146 patients with adenocarcinomas of the esophagus (n=44), gastric cardia (n=28), body (n=24) and antrum (n=50). IHC positivity, as defined by an immunoscore 2+ or 3+ using a modified scoring system, was present in 17 cases with the SP3 antibody, and in 24 cases with 4B5. FISH/SISH showed identical results in 40 cases carried out when any immunoreactivity in either antibody was detected. 100% of SP3-IHC-positive cases, and 92% of 4B5-IHC-positive were amplified. The negative predictive value of SP3 and 4B5 (immunoscores 0/1+) was 77% and 95%, respectively. Heterogenous HER2-positivity with partial staining/amplification was present in 73% of the adenocarcinomas, occasionally with only a tumor area of 10-20% showing positivity. HER2-amplification was present in 27% of esophageal and 18% in gastric cardia carcinomas (resulting in 24% amplification of tumors of the esophago-gastric region). In the distal stomach only 7% HER2-amplification was seen.

Conclusions: HER2 amplification is present in a significant proportion of esophago-gastric region adenocarcinomas (24%) but at a much lower rate in the distal stomach (7%). Both rabbit monoclonal antibodies SP3 and 4B5 can be used for initial screening for possible amplification though the 4B5 antibody has the highest negative predictive value. FISH and SISH yields identical results. The SISH assay offers a easy and fast assessment. The possible role for HER2 as a predictive test for trastuzumab therapy in gastric cancer will be established in the near future.

555 Gastric Foveolar Type Dysplasia Is Common Adjacent to Esophageal Invasive Adenocarcinoma

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Background: Adenocarcinoma in the distal esophagus results of a metaplasia - dysplasia – carcinoma sequence. While Barrett esophagus is an admixture of gastric and intestinal type epithelium, it is long held that only the intestinal phenotype is at risk for neoplastic transformation. The aim of this study was to examine the imunophenotype of dysplasia in the tubular esophagus adjacent to invasive adenocarcinoma.

Design: Sixty six (66) consecutive esophagogastrectomies for lower esophageal adenocarcinoma (N=9) or adenocarcinoma crossing the gastroesophageal junction (N=57) entered into the Australian esophageal cancer study from 6/2001 - 12/2006 were reviewed. The presence of background dysplasia in the tubular esophagus in continuity with the invasive tumour,overlying submucosal glands was recorded. Dysplasia was categorised morphologically into 3 patterns. 1) Gastric foveolar type (FT) characterised by cuboidal to columnar cells with pale clear cytoplasm and hyperchromatic round to oval nuclei, 2) Adenomatous type (AT) - composed of columnar cells with hyperchromatic penicillate pseudostratified nuclei and dense eosinophilic cytoplasm

and 3) Mixed type (MT) showing cytological features intermediate between these two patterns or an admixture (>10%) of the two patterns. Immunohistochemical reactions for p53, Ki 67, gastric foveolar differentiation (MUC5AC) and intestinal differentiation (MUC2, CDX2 and Villin) were evaluated in each case with dysplasia.

Results: Dysplasia (ranging from 0.5mm to >50mm in extent) was identified in 36 of the 66 specimens. FT dysplasia was seen in 19 (53%), AT dysplasia in 10 (28%) and MT dysplasia in 7 (19%) cases. MUC5AC was frequently positive in FT (P=0.07) but MUC2, Villin and CDX2 were negative (all P<0.005). By contrast, AT was frequently positive for MUC2, Villin and CDX2 (all P<0.005).but less frequently positive for MUC5AC (P=0.20).

Table of Immunohistochemistry results % positive

	P53	Ki67	MUC2	MUC5AC	CDX2	VILLIN
FT	11	100	0	84	0	0
AT	40	100	70	40	70	60
MT	57	100	43	43	57	71

Conclusions: Dysplasia in the lower esophagus associated with invasive adenocarcinoma often displays morphologic and immunophenotypic features of gastric foveolar differentiation. There is good concordance between morphologic classification and immunohistochemical phenotyping of the dysplasia patterns.

556 Collagenous Gastritis – A Clinicopathological Study of Nineteen (19) Cases

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Background: Collagenous gastritis (CG) is a condition characterised by thickening of the subepithelial collagen band to >10µm and variable inflammation in the lamina propria. Fewer than 25 cases have been reported. Clinically two subgroups are recognised 1) Pediatric patients who have isolated CG and iron deficiency anaemia (IDA) and 2) Adults who often have a pan enteric collagenous process associated with chronic diarrhea.

Design: Cases were retrieved from 4 institutions. Morphological features including collagen band thickness, characteristics of inflammatory infiltrate, eosinophils per 400x high power field (HPF), intraepithelial lymphocytes (IEL)/100 epithelial cells (EC) and presence of H-pylori were recorded. Follow up biopsies and clinical details including symptomatology and co-morbidities were reviewed.

Results: 19 patients [13 females, 6 males; age range 11-89 years (median 57 years); 3 pediatric] were included. 13 patients presented with IDA and 5 (with associated collagenous colitis (CC)) presented with chronic diarrhoea. Both sites were involved in 11 cases, the antrum only in 2 cases and the corpus only in 2 cases. Maximum thickness of the collagen band was 15-145µm (mean 68µm). Lamina propria chronic inflammation was moderate in 13 cases and severe in 6. Neutrophils were present in 10 cases (with erosion in 3). Eosinophil density >20 per HPF was identified in 13 cases. IEL>25/100 EC were present in 6 cases. H.pylori were identified in 1 case. No atrophy or intestinal metaplasia was noted. 11 patients had biopsies from other GIT sites. 8/11 (adults) had CC; 6/8 (adults) had collagenous ileitis and 3/11 (adults) had collagenous sprue (CS). 1 of 4 patients tested had positive celiac serology. Follow-up in 3 patients showed the condition persisting between 3 to 6 years. Associated conditions included cirrhosis, Graves disease, hypogammaglobulinemia, CLL and achalasia (1 case each).

Conclusions: This large series of CG demonstrates a female predominance and a wide age range at presentation. IDA or chronic diarrhoea is the usual presentation. CG is typically pan gastric in distribution and is characterised by a subepithelial collagen band >10µm thick. Lamina propria inflammation is typical and is often rich in eosinophils. CG can persist for many years. The ileum and colon are often involved in adults, but the duodenum is frequently spared. Like CC, CG is a morphological pattern likely to have a diverse etiology.

557 Eosinophilic Esophagitis (EE): Interobserver Variability (IOV) in a Disease Entity in Which Counting Counts

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Background: EE is a clinicopathologic entity characterized by marked eosinophilic infiltrates of the esophageal mucosa, often associated with upper gastrointestinal (UGI) symptoms and endoscopic abnormalities. A major diagnostic criterion is a density of eosinophils (EOS) above which EE is considered likely. Although eosinophilic density is diagnostically crucial, we are unaware of any data evaluating IOV in the counting of EOS in esophageal biopsies (bxs).

Design: To study the reproducibility of counting EOS and thus the diagnosis (dx) of EE, 2 pathologists (A and B) independently counted the highest number of EOS per high power field (HPF) in esophageal bxs from 252 children with upper endoscopy to investigate UGI symptoms. A diagnostic threshold of 20 or more EOS per HPF was used for the dx of EE. In specimens in which there was a diagnostic discrepancy, a 3rd pathologist (C) counted EOS. To assess agreement (above or below the threshold), 2 statistics were computed: the percentage (%) agreement of cases and kappa coefficients.

Results: Pathologists A and B agreed on the presence or absence of EE in 239 cases, for a 94.8% agreement rate. Their kappa statistic was 0.888. 155 biopsies were counted as less than 20 eos per HPF, and 84 were counted as 20 or more. Of the 13 discrepant bxs, pathologist B contributed the higher count in 12 (92%). Pathologist C agreed with A in 4 cases and with B in 9. In 11 cases, C counted 20 or more EOS per HPF.

Conclusions: IOV is excellent with a kappa of 0.888. Thus, bx interpretation of EE has a very low potential level of IOV. Discrepancies usually result from undercounting of EOS.

558 Ampullary Biopsies: Morphological Features and Useful Biomarker Panel of Adenocarcinoma and Adenoma – A Single Institutional Experience on 273 Ampullary Biopsies

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Background: In ampullary biopsy, diagnosis of adenoma or adenocarcinoma is morphologically challenged due to mix intestinal and pancreaticobiliary epithelium, inflammation, fibrosis and reactive change. It is necessary to find novel biomarkers to differentiating reactive epithelial change from adenoma or adenocarcinoma, Recently, High mobility group A2 (HMGA2) protein, one of the architectural transcription factors, has been found overexpressed in variety of tumors including breast cancer, non-small cell lung cancer, and pancreatic adenocarcinoma. β-catenin and ki-67 are known, useful markers for malignancy. In this study, we systemically analyzed morphological features in 273 ampullary biopsies and used HMGA2, β-catenin and ki-67 as a panel to evaluate malignancy.

Design: 273 ampullary biopsies from 2003-2007 were reviewed. HMGA2, β-catenin and ki-67 immunostains were performed on the cases with marked reactive change, adenomas and adenocarcinomas under proper positive and negative controls. The result was considered as positive if more than 5% epithelial cells showed nuclear HMGA2 or ki-67 staining, cytoplasmic or nuclear β-catenin staining.

Results: Of 273 cases, 55 cases (20%) displayed normal ampullary mucosa, 130 (48%) were benign reactive cases with inflammation, fibrosis, and reactive epithelial change, 62 (23%) were adenomas, 23 (8%) adenocarcinomas and 3 (1%) others. Within 130 benign reactive cases, 84 cases showed inflammation and/or fibrosis, 30 cases had focal pseudostratification and hyperchromasia similar to adenoma, 16 cases showed area of marked cytological atypical and mitosis suspicious for adenocarcinoma. The immunostains on the selected cases showed: 80% adenocarcinomas (4/5), 8% reactive cases (1/12) were HMGA2 positive. None of adenomas (n=12) were positive for HMGA2. Nuclear β-catenin was found in 83% (10/12) adenomas, 100% (5/5) adenocarcinomas and 8% (1/12) reactive cases. Ki-67 showed normal staining pattern in the epithelia in the proliferative zone/crypts in reactive cases, but displayed diffusely increased expression in surface epithelia in all of adenomas and carcinomas.

Conclusions: Morphological diagnosis of ampullary biopsies can be difficult. Our results indicate that the panel of HMGA2, β-catenin and ki-67 would be useful to differentiate benign reactive epithelial change from adenomas/carcinomas. HMGA2 is overexpressed in ampullary adenocarcinoma and may be served as a malignant biomarker for ampullary tumors.

559 Histologic Characterization of Esophagitis Dissecans Superficialis

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Background: Esophagitis Dissecans Superficialis (EDS) is a rare entity characterized by shedding of the esophageal mucosa that may be coughed up or vomited. It has been reported in association with bisphosphonate use and strictures, but most cases remain unexplained. Diagnosis rests on the endoscopic detection of mucosal sloughing, often in a clinical setting of prolonged dysphagia. The histologic correlate is inadequately characterized, and in the absence of defined criteria a descriptive diagnosis is often made. We undertook this study in an attempt to define useful diagnostic histologic criteria for the diagnosis of EDS.

Design: We identified cases by searching : 1) endoscopy database for patients with features compatible with EDS; and 2) pathology database for esophageal biopsies with the keywords “sloughing” and “parakeratosis.” We then cross-searched for EGD, clinical, and histopathologic information.

Results: The EGD search yielded 8 confirmed cases. The pathology search had 12 putative cases: 5 had compatible EGD findings; 6 had strictures and 1 had plaques. Thus, we had 13 confirmed cases, 12 men and one woman (age 48-82). Presentation included: chronic dysphagia (6 pts), upper GI bleeding not from EDS (5), weight loss (1), and asymptomatic (1). By EGD, all had the characteristic “tissue paper” appearance of the esophagus; 6 also had esophagitis (4 w/ grade D); 2 had strictures. None were on bisphosphonate therapy or had bullous skin disorders; 3 had prior surgery (Nissen, Billroth). Histologically, all cases showed sloughing and flaking of superficial squamous epithelium with bullous separation of the layers, parakeratosis, and varying degrees of acute or chronic inflammation. Fungi, identified in 3 cases, were not associated with acute inflammation, likely representing contamination. Follow-up EGD after proton-pump inhibitor therapy showed resolution of esophagitis and EDS in all but one patient. The 7 patients without EDS had similar histology, but without superficial bullous separation; 5 had acute inflammation with debris; the other 2 had acanthosis and parakeratosis; none had fungi.

Conclusions: Histologic features of EDS are similar to biopsies taken from esophageal strictures, and may be confused with candidiasis due to contamination of detached squamous fragments. However, superficial bullous separation seems unique to EDS. Nevertheless, close endoscopic correlation is essential to establishing the correct diagnosis. Both etiology and clinical implications of EDS remain unclear, but it seems to respond to acid-inhibition therapy.

560 *H. pylori*-Negative Gastritis: Effect of Proton Pump Inhibitors

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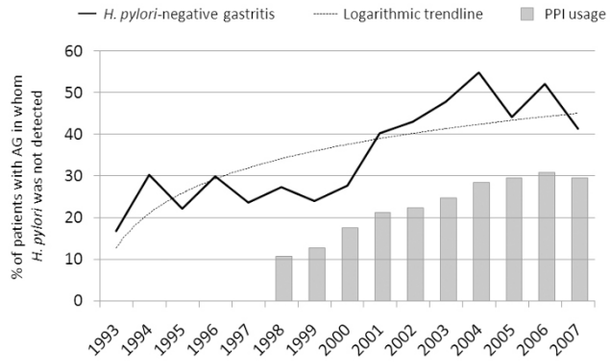
Background: An increasing proportion of gastric biopsies in our veteran population have active or chronic active gastritis (CAG) with no obvious etiology, suggesting that a decrease in detection of *Helicobacter pylori* (*Hp*) organisms may be responsible for this trend. Proton pump inhibitors (PPIs) have limited antimicrobial and anti-inflammatory activity and they alter the gastric distribution of *Hp* populations. This study was designed to test the hypothesis that the increased PPI usage over the past 15 years correlates with the increase in *Hp*-negative CAG.

Design: Patients with gastric biopsies at the Dallas VAMC (1993-2007) were extracted from the surgical pathology database. Diagnoses were tabulated and relevant demographic, clinical, and histopathologic data were collected. Only data from the first gastroscopy were used for patients who had a repeat biopsy during a given year. Patients were stratified in three categories: 1) *Hp* ; 2) CAG without *Hp;* and 3) all remaining patients (normal stomach, inactive, or reactive gastritis). To rule out underdetection due to suboptimal staining or interpretation, we performed IHC on biopsies from all patients with *Hp*-neg CAG and positive *Hp* serology. PPIs, available with restrictions in the VA system since 1993, became widely prescribed since 1998, when electronic records of usage also became available.

Results: Gastric biopsies from 4420 patients were collected and reviewed. Of these, 1112 had *Hp* CAG, including 3 of the 66 *Hp*-seropositive CAG cases (4.5%) found to have *Hp* by IHC. The percentage of *Hp*-negative CAG showed a consistent increasing trend during the study period (Fig. 1), which strongly correlated with the surge in PPI usage ($r = 0.966$; $p < 0.0001$).



Conclusions: In our population, the histoprevalence of *Hp* CAG has sharply declined over the past 15 years, likely due to a combination of a true decrease in the prevalence of *Hp* infection and the masking influence of PPI use on histologic detection. An estimated two thirds of US patients undergoing endoscopy are using PPIs; therefore, the role of histology as the gold standard for the diagnosis of *Hp* ought to be reevaluated.

561 A Multiple Testing Approach for Histological and Molecular Markers for Early Gastric Cancer

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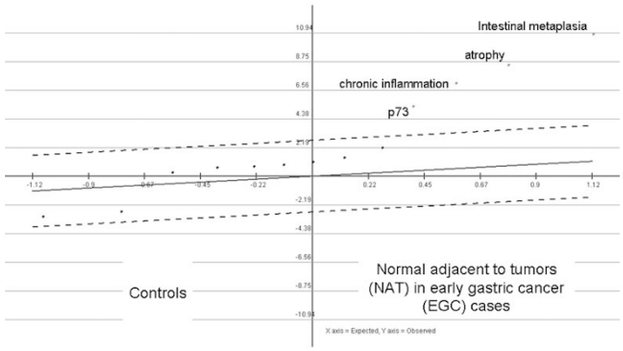
Background: Gastric cancer (GC) is second leading cause of cancer-related death worldwide. The high mortality rate of GC is related with depth of invasion (Early or Advanced GC). To integrate histological and molecular markers in EGC we perfom multiple testing approach.

Design: Ninety one cases of EGC (tumor and normal adjacent mucosa, NAT) and 148 endoscopic biopsies from healthy donors were built into seven TMA blocks containing duplicates 1-mm thickness cores. Five histological (chronic inflammation, PMN, atrophic gastritis, intestinal metaplasia, H. pylori) and seven molecular markers (BRCA1, HSP90, STAT1, FHIT, EGFR, p73 and p16INK4a) were evaluated. For multiple testing approach, Significance Analysis of Microarrays (SAM) was applied. SAM identifies variables with statistically significant changes by assimilating a set of variable-specific t tests. Each variable is assigned a score on the basis of its change in variable expression relative to the standard deviation of repeated measurements for that variable.

Results: Clinical characteristics of EGCs and controls were similar. Chronic inflammation, atrophy and intestinal metaplasia were more frequent in NAT than in controls ($p<0.00001$). H.pylori infection was more frequent in controls ($p<0.001$). Molecular makers in EGC (tumor vs. NAT) were similar except for p53 overexpression in tumors ($p<0.001$). In NAT vs controls only p73 was overexpressed ($p<0.001$). SAM was applied to multiple testing including histological or molecular markers. SAM shows that NAT was characterized chronic inflammation, atrophy and intestinal metaplasia ($p=0.00039$, $p<0.00001$, $p<0.00001$) and overexpression of p73 ($p=0.0061$).



Conclusions: In the statistics of multiple testing, several methods can be applied (the family-wise error rate, the Westfall and Young step-down correction method and the method of Benjamini and Hochberg). Here, we have shown that SAM, a novel method for multiple testing, can be applied to integrate histological and molecular markers. SAM led us to confirm chronic inflammation, atrophy and intestinal metaplasia and identified p73 as a novel marker for EGC. Supported by FONDECYT 1080563.

562 Stat6 Activation and Periostin Expression in Eosinophilic Esophagitis Versus Reflux Esophagitis

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Background: Distinguishing eosinophilic esophagitis (EOE) from reflux esophagitis (RE) with abundant eosinophils is histologically problematic. Prior studies implicated mast cells, IL-5, IL-13, and (at the RNA level) eotaxin-3 and periostin in EOE. In other sites, stat6 phosphorylation and periostin expression are induced by IL-13.

Design: Five subject groups were studied: (pure) EOE – dysphagia, >20 eosinophils maximal/hpf in an esophageal biopsy, and no clinical evidence for reflux; EOE/RE – dysphagia, >20 eosinophils max./hpf, with clinical evidence of reflux (heartburn or abnormal pH probe results); RE-high – clinical evidence of reflux, >20 eosinophils max./hpf and no dysphagia; RE/low – clinical evidence of reflux, <20 eosinophils max./hpf and no dysphagia; and control – healthy volunteers without esophageal problems. There were seven to eight subjects in each group. H&E sections of the esophageal biopsies were reviewed, as well as immunoperoxidase stains for periostin and phospho Tyr-641 Stat6. Review was blinded. Results for the different groups were compared by the nonparametric Mann-Whitney U test.

Results: Phospho-stat6 staining was nuclear. The pure EOE and EOE/RE groups had means of 59% and 45% squamous cell nuclei staining respectively, which were 3.9 and 3-fold higher than the RE/high group (15% nuclei stained). Phospho-stat6 staining was no more than 5% in each subject in the RE/low and control groups. Periostin staining was present in the connective tissue papillae and lamina propria. 7/8 EOE and 5/7 EOE/RE biopsies had abundant periostin staining in the connective tissue papillae. Only 2/7 RE/high, 1/6 RE/low, and 0/7 control biopsies had strong periostin staining. Both the EOE group and the EOE/RE group had significantly more staining for both phospho-stat6 and periostin than each of the other three groups ($p<0.05$, nonparametric Mann-Whitney U test). For both antigens, staining was statistically similar between the EOE and EOE/RE groups, although there were trends for more staining in the pure EOE group. There was more phospho-stat6 staining in the RE/high group (15%) than the RE/low and control groups ($p<0.01$).

Conclusions: As previously shown, high epithelial eosinophil counts did not distinguish EOE from some RE cases. However, periostin and phospho-stat6 staining strongly correlated with dysphagia, with or without coexisting reflux, even among cases with abundant eosinophils. This suggests a common mechanism for patients with EOE and EOE-like disease combined with reflux, involving increased expression and activation of IL-13-related proteins periostin and stat6.

563 Barrett's Esophagus in the Patients with Familial Adenomatous Polyposis

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Background: Familial adenomatous polyposis (FAP) is caused by germ line mutations in *APC* gene. Patients with FAP are at high risk for development of colorectal carcinoma, but small intestinal cancers are the principal cause of death in patients who underwent prophylactic proctocolectomy. Sporadic Barrett's esophagus (BE) and Barrett's adenocarcinoma (BA) are intestinal type lesions of esophagus characterized by an early loss of heterozygosity at the *APC* locus. We hypothesized that patients with FAP are at risk for early development of BE or BA due to the inherited mutations in *APC* gene (haploinsufficiency).

Design: Upper gastrointestinal tract (UGI) biopsies from 36 patients with FAP were reviewed to determine the incidence of BE. The control group consisted of all non-FAP patients undergoing UGI endoscopic examination in our institution in the last 30 months. The difference in expression of Wnt pathway proteins' (APC, β-catenin, E-cadherin and cyclin D1) in BE between BE^+/FAP^+, BE^-/FAP^+ and age-matched BE^+/FAP^- groups was studied using immunohistochemistry. Germline *APC* gene mutations were known in 24 patients.

Results: BE was found in six patients with history of FAP (6/36 or 16% vs. 266/1662 or 16% incidence in the control group). Average age of first diagnosis of BE in FAP patients was 37.8 years vs. 57.5 years in sporadic BE. Both classic FAP and attenuated FAP phenotypes were associated with BE .Two types of germ line mutations in *APC* gene were identified in BE^+/FAP^+ patients: Five patients had 2-base deletion in exon 4 (426delAT) and one patient had 4-base deletion in exon 15 (3202del4). No difference in Wnt signaling pathway proteins' expression was detected between BE^+/FAP^+ and the age matched group (n=10) of patients with sporadic BE (BE^+/FAP^-).

Conclusions: Patients with FAP appear to be equally susceptible to the development of Barrett's esophagus as the general population, indicating that the primary effect of a common pathophysiologic mechanism (gastroesophageal reflux) underlies develoment of BE in FAP and in sporadic cases. However, Barrett's esophagus in FAP patients is detected on average 20 years earlier than in the general population. Although this early development of BE may indicate a predisposing effect of the germline *APC* gene mutations, early detection of BE is likely due to the endoscopic surveillance of FAP patients for the upper GI tract abnormalities.

564 Characterization of Expression of a Novel Smooth Muscle Contractile Protein Smoothelin in Gastrointestinal Tract: Implication in the Pathogenesis of Colonic Inertia

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Background: Colonic inertia (CI) is a frustrating motility disorder not only to clinicians but also to pathologists. Its etiopathogenesis is largely unknown. The aim of this study

was to characterize the expression of smoothelin, a novel contractile protein expressed only by fully differentiated smooth muscle cells, in normal gastrointestinal (GI) tract and to determine if smoothelin is aberrantly expressed in patients with CI.
Design: A total of 67 resections of normal GI tract from patients with dysmotility-unrelated disorders (distal esophagus 3, stomach 4, duodenum 12, ampulla 10, jejunum 7, ileum 11, right colon 10 and left colon 10) and 28 colon resections (16 with terminal ileum) from patients with CI were included in this study. Full-thickness and well-oriented sections were selected for immunostaining for smoothelin. Staining for smooth muscle actin (SMA) was used for comparison.
Results: In controls, strong and diffuse cytoplasmic staining for smoothelin was observed in both inner and outer layers of the muscularis propria (MP) throughout the entire GI tract. In contrast, the muscularis mucosae (MM) were either completely negative or only patchy and weakly stained with the exception of the distal esophagus where the MM was also strongly and diffusely stained. In CI patients, a moderate to marked reduction in smoothelin immunoreactivity was observed in 16 of 28 (57.1%) colon resections exclusively in the outer layer of the MP, compared to normally stained inner layer. Of these 16 cases, a similar reduction in smoothelin immunoreactivity was also observed in 3 (18.8%) ileal resections. SMA stained both inner and outer layers strongly and diffusely in all colon resections. In contrast, a moderate to marked reduction in SMA immunoreactivity, exclusively in the inner layer of the MP, was observed in 9 (56.3%) ileal resections, of which 4 had reduced smoothelin expression in the colon and 2 in both colon and ileum.
Conclusions: Smoothelin is differentially expressed in the MP and MM of the GI tract, which may be of potential utility in cases where the distinction between the two is necessary. Defective smoothelin expression in the presence of essentially normal histologic findings and the preservation of SMA immunoreactivity suggests a role in the pathogenesis of CI.

565 Notch Overexpression in Colorectal Adenocarcinoma (CRC)

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Background: Notch signaling is dysregulated in a variety of cancers, including cervical, head and neck, endometrial, renal, lung and breast carcinomas and hematological malignancies. In intestinal epithelium, Notch signaling via HES1 has been shown to promote the differentiation of enterocytes from intestinal 'stem cells' while inhibiting the differentiation of secretory cells. The prognostic significance of Notch2, Notch3 and Notch4 expression in CRC has not been previously studied.
Design: Formalin-fixed, paraffin-embedded sections from 125 colorectal adenocarcinomas (CRCs) were immunostained by an automated method (Ventana Medical Systems; Tuscon, AZ) using polyclonal rabbit IgG antibodies to Notch-2 (sc-5545), Notch-3 (sc-5593) or Notch-4 (sc-5594); (Santa Cruz Biotechnology, Santa Cruz, CA). Cytoplasmic immunoreactivity was semiquantitatively evaluated based on both intensity and distribution and results were correlated with histologic and prognostic variables.
Results: Overexpression of Notch2 was observed in 43/125 (34%) of CRCs, overexpression of Notch3 was seen in 29/116 (25%), and of Notch4 seen in 27/122 (22%). With all three Notch biomarkers, increasing expression was observed with increasing grade: for Notch2 (17% of grade 1, 32% of grade 2 and 54% of grade 3, p=0.034); for Notch3 (33% of grade1, 17% of grade 2 and 42% of grade 3, p=0.030); for Notch4 (11% of grade 1, 15% of grade 2 and 50% of grade 3, p=0.001). Notch2, Notch3 and Notch4 expression did not correlate with pathologic stage, disease recurrence or overall survival. On multivariate analysis, pathologic stage at diagnosis independently predicted patient survival.
Conclusions: Notch2, 3 and 4 proteins are overexpressed in colorectal adenocarcinomas, and the expression of each biomarker correlated with aggressive tumor histology. Notch2, Notch3 and Notch4 overexpression may be useful prognostic indicators in CRC, and further investigation of these biomarkers appears warranted.

566 Smoothelin Is a Specific Marker for Smooth Muscle Neoplasms of the Gastrointestinal Tract

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Background: Smoothelin is a smooth muscle (SM)-specific cytoskeletal protein exclusively found in differentiated SM cells, unlike other SM proteins (e.g., h-caldesmon, α-smooth muscle actin, desmin, smooth muscle myosin), which are expressed in proliferative (early) stages of SM development and occasionally in other cell types (striated muscle, myofibroblasts, myoepithelial cells, pericytes). Smoothelin has been shown to be expressed predominantly in visceral SM and to a lesser extent in vascular SM. Smoothelin expression in mesenchymal tumors of the gastrointestinal (GI) tract has not previously been evaluated. The purpose of this study was to determine whether immunostaining for smoothelin could help distinguish SM neoplasms from their morphologic mimics, particularly KIT-negative gastrointestinal stromal tumors (GISTs), desmin-positive GISTs, and desmoid fibromatosis.
Design: In total, 107 spindle cell tumors of the GI tract, abdominal cavity, and retroperitoneum were retrieved from consult and surgical pathology archives, including 36 GISTs (6 KIT-negative; 9 desmin-positive), 12 GI leiomyosarcomas (LMS), 9 GI mural leiomyomas, 13 colorectal leiomyomas of the muscularis mucosae, 9 gastric schwannomas, 12 inflammatory myofibroblastic tumors (IMT), 7 cases of mesenteric desmoid fibromatosis, and 9 dedifferentiated liposarcomas (DDLPS). Immunostaining for smoothelin (Chemicon; clone R4A; 1:800 dilution) was performed on all cases. Cytoplasmic and nuclear staining was recorded.
Results: Diffuse, predominantly cytoplasmic expression of smoothelin (>75% of tumor cells) was present in all 22 (100%) benign GI SM tumors. In contrast, only 2 (17%) GI LMS showed cytoplasmic staining for smoothelin. None of the GISTs, desmoid tumors, IMTs, schwannomas, or DDLPS showed cytoplasmic reactivity for smoothelin. Interestingly, 7 (58%) GI LMS and 6 (17%) GISTs (all with an epithelioid component) showed multifocal, exclusively nuclear staining for smoothelin. Nuclear expression of smoothelin was not detected in any of the other tumor types examined.
Conclusions: Diffuse cytoplasmic staining for smoothelin is highly sensitive and specific for benign leiomyomas of the GI tract. Aberrant nuclear only expression is common in GI LMS and may also be seen in epithelioid and mixed-type GISTs. These findings suggest that the extent and pattern of smoothelin expression may help differentiate between benign and malignant SM tumors of the GI tract, and may also be useful in distinguishing GI leiomyomas from KIT-negative and/or desmin-positive GISTs.

567 Surface Mitoses Are a Predictor of Cancer Progression in Barrett's Esophagus

DP Coco, A Srivastava, CA Sanchez, X Li, D Cowan, BJ Reid, PL Blount, RD Odze. Brigham and Women's Hosptial, Boston, MA; Dartmouth Hitchcock Medical Center, Lebanon, NH; Fred Hutchinson Cancer Center, Seattle, WA; University of Washington, Seattle, WA.
Background: At present, dysplasia is the most reliable biomarker of cancer progression in patients with Barrett's esophagus (BE). However, dysplasia interpretation suffers from a high degree of interobserver variability, and only a small proportion of BE patients develop this morphologic alteration. Cell cycle abnormalities and increased cell proliferation have been linked to cancer in BE. In a previous preliminary study, we reported an association between the presence of surface mitoses and progression to adenocarcinoma in BE. The aim of this study was to evaluate the prognostic significance of mitoses in a large prospective cohort of BE patients with long-term follow-up.
Design: 1752 routinely processed mucosal biopsies from 101 BE patients (M/F ratio: 85/16, mean age: 63.3 years, mean BE segment length 5.4 cm), followed for a mean of 85.5 months (5.8-158.4 months), all of whom had a baseline "index" endoscopy between 1995 and 1999 and at least 1 follow-up endoscopy, were included. The development of adenocarcinoma was the primary outcome variable. All biopsies were evaluated, in a blinded fashion, for number of mitoses within dysplastic and non-dysplastic epithelium. Data was analyzed using a Cox regression model to account for follow-up intervals and censored data.
Results: A strong positive correlation was noted between the presence of surface mitoses (both typical and atypical) and the development of adenocarcinoma ($p<0.0001$, hazard ratio [HR]: 5.0, 95% CI 4.0-6.3). The presence of atypical mitoses (surface and crypt) was also a predictor of progression ($p<0.0001$, HR: 2.9, 95% CI 2.1-4.3). Specifically, within this group, atypical surface mitoses had the strongest correlation ($p<0.0001$, HR: 3.6, 95% CI 2.2-5.9). No significant correlation was noted between crypt mitoses and the development of adenocarcinoma.
Conclusions: Surface epithelium mitoses are a valuable morphologic biomarker of progression to cancer in BE. This is consistent with the previously well-known association between increased proliferation, cell cycle abnormalities and cancer progression in BE. (Supported by NIH P01CA91955).

568 Tumour Budding in TI and T2 Node-Negative (Dukes A) Colorectal Carcinoma

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Background: Tumour budding has been shown to be a strong, independent, and reproducible prognostic marker of patient outcome in T3N0 colorectal carcinoma by Wang et al (Am J Surg Path, in press). The significance of tumour budding in T1N0 and T2N0 tumours has not been widely studied.
Design: One hundred and twenty-one T1N0 and T2N0 colorectal carcinoma cases were identified from the database at this institution over a fifteen-year period (January 1993-July 2008). A subset of these included polypectomy specimens with carcinoma arising in polyps (n=28). Synchronous tumours, ulcerative colitis and familial adenomatous polyposis associated tumours were excluded. Haematoxylin & eosin stained sections from each case were examined for tumour budding using the rapid bud count method described previously (five areas examined at 200x per slide). Additional prognostic parameters studied included lymphovascular invasion, tumour margin, depth of invasion, and tumour differentiation. Patient outcome was evaluated and correlated with these findings.
Results: Eighty-five (70%) of carcinomas had low and thirty-six carcinomas (30%) had high bud scores. High budding was associated with an infiltrative margin ($p=<0.0001$). High budding was not seen in the six cases of intramucosal carcinoma. High budding was associated with a greater depth of invasion ($p=0.03$), present in 8/42 (19%) of T1 carcinomas and 28/72 (39%) of T2 carcinomas.The median follow-up of patients was 2.03 years (range 0-12.4 years). Cancer deaths were observed in 7% of patients in the low budding category versus 8.6% of patients in the high budding category (p=0.66).
Conclusions: High tumour budding was identified in 30% of Dukes A colorectal cancers. High tumour budding is associated with a greater depth of invasion and with an infiltrative margin. The presence of tumour budding did not predict a poorer prognosis in this series. A study of a larger number of cases with a longer duration of follow-up may be necessary to investigate the value of tumour budding as a predictor of survival in the T1N0 and T2N0 setting.

569 Histologic Evaluation of Excised Pouch Specimens in Distinguishing Crohn's Disease of the Pouch (CDP) from Chronic Antibiotic-Refractory Pouchitis (CARP)

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Background: Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the treatment of choice in pts with presumed ulcerative colitis/ indeterminate colitis

who require surgical excision. IPAA is generally contraindicated in pts with Crohn's disease, due to a higher risk of pouch failure. Some IPAA pts develop poorly functioning pouches requiring excision; common causes of pouch failure include CDP and CARP. The aim of this study was to determine whether histologic features could reliably distinguish between pts with a clinical diagnosis of CDP and CARP.

Design: Our institutional pouchitis database was used to identify pts with a clinical diagnosis of CDP or CARP who underwent pouch excision. The diagnosis of CDP was based on a combined assessment of clinical, endoscopic, radiographic, and histologic features as well as prior response to anti-TNF therapy. CARP was defined as failure to respond to a 4-week course of a single antibiotic, requiring prolonged therapy of ≥ 4 weeks. We evaluated 16 cases of CDP and 10 cases of CARP for neutrophil infiltration, ulceration, villous blunting, crypt distortion, and mononuclear cell and eosinophil infiltration of the lamina propria (0-3+). Cases were also evaluated for the presence of granulomas, pyloric gland metaplasia, intraepithelial lymphocytosis, transmural inflammation, and fistulae.

Results: CDP group included 9 males and 7 females, age 23-50 years (mean 38.0 years). CARP group included 4 males and 6 females, age 44-70 years (mean 57.2 years).

	CDP (N=16) Mean score, 0-3 scale	CARP (N=10) Mean score, 0-3 scale	P value
Neutrophilic infiltration	1.12	1.6	0.27
Ulcer	2.19	1.9	0.51
Villous blunting	1.87	1.05	0.76
Crypt distortion	1.37	1.6	0.58
Mononuclear infiltration	1.5	1.9	0.25
Eosinophils	1.37	0.71	0.67

	CDP (N=16)	CARP (N=10)	P value
Granulomas	3/16	0/10	0.14
Pyloric gland metaplasia	8/16	5/10	1.0
Intraepithelial lymphocytes	0/16	0/16	NA
Transmural inflammation	2/16	3/10	0.27
Fistula	6/16	0/10	0.049

Conclusions: Only fistulae were associated with a clinical diagnosis of CDP. Although granulomas were found only in CDP, this feature was seen in very few pts and was not significantly associated with CDP. All other histologic features, including transmural inflammation, were not useful in distinguishing CDP from CARP.

570 Junctional Adhesion Molecule-A Expression in Crohn's Disease and Ulcerative Colitis

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Background: Junctional adhesion molecules (JAMs) are transmembrane components of tight junctions that have been implicated in regulating leukocyte migration in tissues. Previous studies have demonstrated that one JAM protein family member JAM-A regulates permeability and inflammation in the intestine of a knockout mouse. Furthermore, a recent study reported downregulation of JAM-A expression within colonic epithelial cells in areas of active intestinal inflammation in a mouse model. Despite these reports, detailed studies of JAM-A expression in human inflammatory bowel disease (IBD) tissues have not been performed.

Design: Fifty resected colons (20 Crohn's disease, 22 ulcerative colitis, 8 non-IBD controls) were examined. Quiescent and active areas were selected from each IBD case. Paraffin-embedded tissues were examined for JAM-A expression using a specific mAb1H2A9 and DAKO Envision+dual link system. This method employs an HRP labeled polymer with prior heat-induced antigen retrieval. Epithelial, endothelial and stromal expression of JAM-A were evaluated semi quantitatively.

Results: We observed upregulation of JAM expression in the subapical tight junction regions of intestinal crypts in regions with active inflammation, particularly in areas adjacent to ulcers. Findings in ulcerative colitis samples were similar to those in Crohn's disease. JAM-A staining was consistently more intense in areas of active inflammation than in quiescent areas. Furthermore, expression of JAM-A in quiescent areas was similar to that in the control group that consisted of normal colonic tissue from non-IBD cases. In addition, we observed increased staining with mAb 1H2A9 in stromal cells within active areas of inflammation and in endothelial cells. A marked increase in staining was also observed focally in the vasculature on the serosal side of intestinal sections of actively diseased IBD samples.

Conclusions: Our findings suggest that JAM-A expression in inflammatory bowel disease is upregulated during the active phase of the disease, which may play an important role in regulation of leukocyte migration and mucosal permeability. Whether these changes in JAM-A expression are directly or indirectly linked to disease pathogenesis requires further study.

571 Morphoproteomic Confirmation of an Activated NF-Kappa B Pathway in Colorectal Adenocarcinoma

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Background: The upregulation of interleukin 8 (IL 8) has been reported to be associated with induction, progression and metastatic potential of colorectal cancer. IL-8 is also a known transcriptional target of nuclear factor (NF)-kappa B and is overexpressed in a number of malignancies. In this study, we investigated the constitutive activation of the NF-kappa B pathway in colorectal adenocarcinoma (CAD) versus non-neoplastic colonic epithelium.

Design: A Tissue microarray with 30 primary colonic adenocarcinoma and 20 non-neoplastic colonic mucosa was assembled and immunostains were utilized for detection of IL-8, phosphorylated (p)-NF-kappaBp65 (Ser 536) and glutathione S transferase(GST)-pi. Chromogenic signal and cellular localization were assessed by bright field microscopy and the intensity of these signals was scored as (0-3+).

Results: Strong (2+ to 3+) IL-8 expression was observed in (28/30)97% of CAD tissues and the signal was predominantly perinuclear while non-neoplastic epithelium was essentially negative. p-NF-kappa Bp65 and GST-pi were expressed in all (100%) cases of cancer as well as non-neoplastic colonic epithelium tissue; however, the immunostaining was stronger (2+ to 3+) in CAD versus weak to 1+ in non-neoplastic tissue. The GST-pi localization was cytoplasmic and the p-NF-kappaBp65 was predominantly nuclear.

Conclusions: Morphoproteomic analysis reveals the activation of the NF-kappa B pathway in colorectal adenocarcinoma as evidenced by overexpression of NF-kappa Bp65 (Ser536) with nuclear translocation and parallel increases in expression of IL-8 and GST-pi. The perinuclear localization of IL 8 provides supportive evidence of the activation of NF-kappa B. This study confirms the activation of NF-kappa B pathway in the pathogenesis of CAD leading to transcriptional activation of IL-8 and GST-pi genes.

572 Down-Regulation of RKIP Predicts a Worse Outcome in Dukes B Colorectal Carcinoma. Is This Down-Regulation Due to Hypermethylation?

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Background: Raf Kinase Inhibitor Protein (RKIP) is an inhibitor of the Ras-Raf-MAPK pathway. Down-regulation has been shown to be important in a number of common cancers, including colorectal carcinoma (CRC). Dukes B CRC represents a significant post-operative management challenge and additional biomarkers that give further information on prognosis would be useful. The mechanism of RKIP down-regulation is controversial. Some authors have suggested promoter hypermethylation as the mechanism. Pyrosequencing is recognised as the gold-standard in methylation analysis. Here we use pyrosequencing to examine RKIP promoter hypermethylation.

Design: A tissue microarray consisting of 211 cases of Dukes B CRC with matched normal tissue was stained with an antibody for RKIP. Expression level was scored using a semi-quantitative scoring system which divided samples into 3 groups (Negative, Weakly Positive and Strongly Positive). RKIP expression was correlated with survival and with traditional clinico-pathological parameters. Pyrosequencing was used to examine RKIP promoter methylation in 30 CRC and matched normal tissues.

Results: 97 patient samples showed strongly positive expression of RKIP, 100 showed weakly positive expression and 14 were negative. RKIP expression levels correlated significantly with survival (p=0.007) in this group of Dukes B CRC patients. Patients with the highest levels of RKIP expression had the best prognosis and those with the lowest levels of RKIP expression had the worst prognosis. This correlation was independent of traditional clinico-pathological parameters such as T-Stage and lympho-vascular invasion (LVI). Of the 30 CRC samples only one showed hypermethylation of the RKIP promoter as measured by pyrosequencing.

Conclusions: RKIP expression levels can sub-stratify patients with Dukes B CRC by prognosis. Importantly this difference was independent of whether the tumour was T3 or T4 and other clinico-pathological parameters such as LVI. RKIP could be a very useful addition to current methods for selecting high-risk Dukes B CRC patients who may benefit from closer post-operative monitoring and potentially adjuvant therapy. Promoter hypermethylation does not appear to be the mechanism of RKIP down-regulation and further study is required to identify the mechanism.

573 Gastric Atrophy Diagnostic Accuracy: Comparison of OLGA with Baylor System

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Background: The risk of developing intestinal type gastric cancer is related to the extent of atrophy. Two histopathologic based systems (OLGA, and Baylor) have been introduced as an index of gastric atrophy. OLGA incorporates the updated Sydney System (SS) biopsy protocol (3 antral and 2 mid-corpus biopsies along the lesser and greater curvature) and a histopathology scoring system averaging scores by region then combines these scores. Baylor uses Baylors biopsy protocol (which uses SS but adds 2 additional distal corpus biopsies) and scores the antrum and corpus independently. Antral atrophy stage is an average score, but corpus atrophy stage is independent of antral atrophy, independent of individual reading in each biopsy but dependent on location. As corpus atrophy starts at the incisura and extends in continuity proximally and towards the greater curve, atrophy in a distal biopsy is early and atrophy in the most proximal location is advanced. This study compares the diagnostic accuracy of each system.

Design: Biopsies were examined from 127 patients who had endoscopy with biopsy using Baylors protocol. OLGA and Baylor atrophy scores were compared with serum pepsinogen I/II ratio as a marker for corpus atrophy and serum gastrin level as a marker for antral atrophy.

Results: Baylor System of scoring corpus atrophy by location was superior to OLGAs average score from all biopsies ($p < 0.0001$). Incorporating distal corpus biopsies improved corpus atrophy recognition ($p < 0.0001$) but did not affect the overall OLGA stage ($p = 0.6$); OLGA was heavily influenced by antral atrophy score, enough to show a positive (not negative) correlation with serum gastrin levels. Corpus atrophy was identified in 57 (36%) patients using Baylor compared to 33 (26%) using OLGA ($< p = 0.001$). Baylor System showed that, in the antrum, the extent of intestinal metaplasia, and not just antral gland atrophy, correlated best with decreasing fasting serum gastrin levels. Patients with autoimmune gastritis were unrecognized in OLGA but readily identified using Baylor.

Conclusions: In following the SS biopsy site and scoring system recommendations, OLGA systematically underestimated the presence of corpus atrophy and thus cancer risk. In addition, diagnostic information was lost in lumping comparative measures (antral versus corpus atrophic gastritis). Studies for cancer risk should use the Baylor system (biopsy protocol and scoring system).

574 Expression of Lgr5 in Colorectal Carcinoma Correlates with That of β-catenin: A Tissue Microarray Study

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Background: Lgr5 is a member of the G protein super-family and was shown recently to be a stem cell marker for cells with intestinal differentiation. Its over-expression has been demonstrated in colorectal carcinomas (CRC) but the underlying mechanisms remain unknown. The aim of this study was to investigate if Lgr5 was over-expressed in CRC in Chinese patients and if so, whether Lgr5 expression was correlated with β-catenin for its involvement in CRC development through the WNT pathway.

Design: The study was carried out on a tissue microarray that consisted of 102 CRC (M:F=55:47), 18 (M:F=10:8) colon adenoma (CA), and 11 (M:F= 8:3) colon normal mucosa (CNM) cases. The tissue core was duplicated for each case. Immunostains were performed with a standard EnVision method with primary antibodies against Lgr5 (Lifespan Biosciences, 1:400), β-catenin (Zymed Labs, 1:200), and p53 (Zymed Labs, 1:300) antigens. Immunoreactivity of cells was double-blindly semi-quantified by two pathologists and the data were compared with the Chi-square and Spearman rank correlation tests.

Results: Lgr5 immunoreactivity was observed only in single cells in the base of normal crypts but strong in 28% CA, and significantly higher in 54% ($p=0.016$) CRC cases. In CNM, CA, and CRC, β-catenin immunoreactivity was seen in 25%, 27%, and 81% cases, respectively, in contrast to 0, 0, and 40% for p53 immunoreactivity, respectively. In CRC, Lgr5 expression was more common in women than men ($p<0.0001$), and positively correlated with β-catenin expression ($p<0.001$), but not with patient ages, tumor sizes, nodal status, TNM stages, and p53 expression.

Conclusions: Intense Lgr5 expression was common in CRC, more frequent in female than male patients, and positively correlated with β-catenin expression. The results suggest that Lgr5 may play an important role in CRC development in Chinese patients through the WNT pathway, which remains to be further investigated.

575 Nucleolin Expression in Colorectal Adenocarcinomas: Higher Nucleolin Expression Is Associated with Better Survival in Stage III Colorectal Cancers

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Background: Nucleolin is a ubiquitous, nonhistone nuclear phosphoprotein that is present in nucleolus and is involved in the synthesis and maturation of ribosomes. Recent studies have shown that nucleolin is over-expressed in highly proliferative cells and is involved with gene expression. The expression of nucleolin and its significance in colorectal adenocarcinomas have not been studied. The aim of this study was to examine the expression of nucleolin in colorectal adenocarcinomas and correlate with clinicopathological features and patient's survival.

Design: 140 stage II and 80 stage III colorectal adenocarcinomas were retrieved from the files of MD Anderson Cancer Center between 1990 and 1998. A tissue microarray consisting of duplicate 1 mm cores of tumor and paired colorectal mucosa were stained with an anti-nucleolin antibody by immunohistochemistry. The nucleolar staining for nucleolin was scored quantitatively using the Ariol Image Analysis System (Genetix Limited, UK) and expressed as ratio of nucleolar staining per total nuclear area in mucosal epithelial cells and neoplastic glands. The statistical differences between means were analyzed by T-test and survivals by Kaplan and Meier log-rank method.

Results: The expression level of nucleolin was higher in colorectal adenocarcinomas (4.49 ±6.14) than in paired colorectal mucosa (0.92 ±1.90, $p=0.001$). The nucleolin levels were similar in stage II carcinomas (4.50 ±6.10) and in stage III carcinomas (4.46 ±6.2, not significant). Among patients who had stage III colorectal carcinoma, the quarterlies of tumors with the highest nucleolin expression (>6.72) had better survival (110.5 ± 52.1 months) than the remaining three quarterlies (83.2 ± 48.4 months, $p=0.018$).

Conclusions: Nucleolin is over-expressed in colorectal adenocarcinoma. Tumors with the higher quarterlies of nucleolin expression have better survival in stage III colorectal adenocarcinoma. Nucleolin may be a prognostic marker for predicting clinical outcome in stage III colorectal adenocarcinoma.

576 Expression of Insulin-Like Growth Factor II mRNA-Binding Protein 3 (IMP3) in Human Esophageal Adenocarcinoma and Its Precursor Lesions

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Background: Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is an oncofetal protein highly expressed in fetal tissue and malignant tumors such as endometrial carcinomas, renal cell carcinomas and melanoma, but only rarely within adult benign tissues. The prevalence and significance of IMP3 in esophageal adenocarcinoma (EAC) and its precursor lesions including distinctive type Barrett's mucosa (intestinal metaplasia, BM), and dysplasia are largely unknown.

Design: Samples from 155 cases of esophageal adenocarcinoma, 21 cases of esophageal columnar dysplasia (12 high grade dysplasia and 9 low grade dysplasia cases), 31 cases of BM without dysplasia and 122 cases of non-neoplastic esophageal mucosa without dysplasia or BM within formalin-fixed paraffin-embedded tissue microarray blocks were examined. Cases with preoperative treatment were excluded. Tissue microarrays were stained with mouse monoclonal anti-IMP3 antibody (Dako, 1:80) with adequate positive and negative controls. The percent (0-100%) and intensity (1-3+) of positive cytoplamic and/or membranous IMP3 staining cells were determined.

Results: A subset of EAC cases (103, 66%) showed positive cytoplasmic and membranous IMP3 staining. Sixty percent of poorly differentiated EAC showed strong IMP3 staining (2-3+) compared to well and moderately differentiated EAC (48% and 45% respectively). In addition poorly differentiated EAC showed statistically significant higher IMP3 expression compared to well differentiated EAC ($p<0.005$), Twenty-four percent of dysplasia cases were positive for IMP3. Four low grade dysplasia case showed positive (1+ in <15% of lesional cells) IMP3 staining and 1 high grade dysplasia cases showed positive diffuse 2+ IMP3 staining. In contrast, only 1 BM case showed positive diffuse 2+ IMP3 staining and no IMP3 staining was observed in any of the non-neoplastic esophageal mucosa without dysplasia or BM.

Conclusions: This study shows that IMP3 is overexpressed in esophageal adenocarcinomas, and is expressed significantly in lower frequency of pre-neoplastic lesions and are negative in non-preneoplastic esophageal mucosa. Furthermore, statistically significant differences in level of IMP3 expression between well differentiated and poorly differentiated EACs, suggest that IMP3 over-expression may have potential prognostic implications in EAC.

577 Up-Regulation of REGγ Is Associated with Human Gastric Adenocarcinoma: A Study of 216 Cases

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Background: REGγ (PA28γ) is an activator of the 20S proteasome, which plays roles in apoptosis and mitotic progression. It has been shown to be overexpressed in poorly differentiated thyroid papillary carcinoma, colorectal cancer, and uterine leiomyosarcomas. In this study, we evaluated the expression and prognostic correlation of REGγ in gastric adenocarcinoma (GAC).

Design: Samples from 216 cases of gastric adenocarcinoma and 16 cases of non-neoplastic gastric tissues within formalin-fixed paraffin-embedded tissue microarray blocks were examined. Cases with preoperative treatment were excluded. Tissue microarrays were stained with REGγ antibody with colorectal cancer cells as positive control and internal stromal cells as negative control. The percent (0-100%) and intensity (1-3+) of unequivocal positive REGγ nuclear staining cells were determined and a composite score (CS: product of intensity and percent of positive cells, ranging 0 to 300) was calculated. REGγ expression was also correlated with patient survival data.

Results: GAC cases showed higher intensity and percent of cells with positive REGγ nuclear expression (average CS = 74), compared to non-neoplastic gastric controls (average CS = 40). 76% of GAC cases showed positive REGγ nuclear staining (intensity ≥1+). High REGγ overexpression cases showed better 10 year and 15 year survival (53% and 51% respectively) compared to low (rare 1+ staining) or negative REGγ expression cases (10 year and 15 year survival of 38% and 33% respectively), $p<0.05$.

Conclusions: The present study shows REGγ is over-expressed in gastric adenocarcinoma. It demonstrates that up-regulation of REGγ is associated better long term survival. These findings suggest that REGγ has a vital role in carcinogenesis and is a potential useful prognostic marker for GAC.

578 CDK8 Expression Is Associated with β-Catenin Activation and Poor Outcome in Colon Cancer

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Background: Molecular alterations in the Wnt/β-catenin pathway are a key tumorigenic event in colon cancer. We have recently identified CDK8, a cyclin-dependent kinase, as a colon cancer oncogene that is necessary for β-catenin activity (Firestein R., et al. Nature 2008). We tested the hypothesis that colon cancers with CDK8 expression have distinct clinical and molecular attributes that may impact future therapeutic directions.

Design: Utilizing 483 colorectal cancers (stage I-IV) in two independent prospective cohort studies, CDK8 expression was detected in 341 (71%) tumors by immunohistochemistry. Cox proportional hazard models computed hazard ratios (HRs) of colorectal cancer-specific and overall mortalities, adjusted for patient characteristics and tumoral molecular features, including β-catenin, p53, p21, p27, cyclin D1, CIMP (CpG island methylation phenotype), MSI (microsatellite instability), LINE-1 methylation, KRAS, BRAF and PIK3CA.

Results: CDK8 expression was significantly associated with female ($p=0.007$), high β-catenin activity ($p=0.0003$), fatty acid synthase (FASN) expression ($p=0.0003$) and p53 expression ($p=0.007$), which persisted in multivariate logistic regression analyses. Compared to patients with CDK8-negative colon cancers, those with CDK8-positive colon cancers experienced a high cancer-specific mortality (HR 1.57; 95% CI, 0.94-2.61), which became statistically significant in multivariate analysis (adjusted HR 2.18; 95% CI, 1.19-3.99). Similar findings were observed for an overall mortality (adjusted HR 1.53; 95% CI, 1.00-2.34). The association of CDK8 expression with a high mortality appeared to persist in various strata of clinical or molecular features, including tumor stage.

Conclusions: CDK8 expression in colon cancer is associated with high β-catenin activity and poor prognosis.

579 Overexpression of Transcription Intermediary Factor 1 γ (TIF1γ) Is Associated with Low Grade Gastric Adenocarcinomas and Is Independent of SMAD-4 Depletion in TGFβ Pathway Dysregulation

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Background: Transcription Intermediary Factor 1 gamma (TIF1γ) is a competitor of SMAD4 for binding to receptor SMADs downstream of TGFβ receptor activation. It has been shown that about 30-50% of gastric cancers show SMAD4 depletion; however, it is unknown whether abnormal expression of TIF1γ may also contribute to dysregulation of TGFβ signaling pathway in gastric cancers.

Design: Forty-six consecutive gastric cancer resection specimens were retrieved from the NYU Medical Center pathology database. The type and degree of differentiation of

cancer was determined by two independent pathologists. Four micron-thick paraffin-embedded tissue sections were cut and immunostained for TIF1γ, SMAD4 and TGFβII receptor using a Ventana automated immunostainer. Staining intensities were graded as 1+ (baseline expression in normal epithelium), 2+ (moderate) or 3+ (high expression). Abnormal expression in more than 30% cancer cells was considered to be positive.

Results: TIF1γ overexpression is seen in 27/46 (59%) of gastric cancers. It is more frequently associated with intestinal type of gastric adenocarcinoma (19/26; 73%) and with well and moderately differentiated cancers (22/30; 73%) than with diffuse type (3/11; 27%) or poorly differentiated gastric cancers (5/16; 31%). SMAD4 depletion is seen in 19 of 46 (40%) cases. In contrast to TIF1γ overexpression, SMAD4 depletion is more commonly observed in poorly differentiated (69%) and diffuse type gastric cancers (73%) than in well and moderately differentiated (27%) or intestinal type (23%) cancers. TGFβ overexpression is seen in 32/46 gastric carcinomas (70%) and is more common in intestinal type (88%) and in well and moderately differentiated cancers (83%) than in diffuse type (36%) or poorly differentiated gastric cancers (44%). Sixty-eight percent of cases with increased TGFβ and TIF1γ expression show unaffected SMAD4 levels (15/22) and 32% show depleted SMAD4 levels (7/22).

Conclusions: Overexpression of TIF1γ parallels TGFβ2 overexpression in gastric cancers. In contrast to SMAD4 depletion, TIF1γ is commonly associated with low grade and intestinal type cancers. There is no association between TIF1γ overexpression and SMAD4 depletion in the majority of cases. These findings suggest that TIF1γ overexpression may play an important role in development of low grade and/or intestinal type gastric cancer consequent to dysregulation of TGFβ signaling pathway.

580 Immunohistochemical Detection of Sporadic and Hereditary Colorectal Carcinomas with Defective DNA Mismatch Repair

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Background: Identification of colorectal carcinomas (CRCs) with defective DNA mismatch repair (MMR) is of great clinical relevance. Aim of the present study was to precisely determine the role of immunohistochemistry as a screening test for the detection of these tumors.

Design: A consecutive series of 330 CRCs was included in the study. Expression of MMR proteins (MLH1, MSH2, MSH6 and PMS2) was evaluated by immunohistochemistry and microsatellite instability (MSI) by a fluorescence PCR method using mononucleotide (BAT26, BAT25 and BAT40) and dinucleotide markers. MLH1 promoter methylation was determined by methylation specific PCR.

Results: Deficit of MMR was observed in 51 tumors (MMR-D, 15.5%), whereas the remaining 279 CRCs showed normal protein expression and microsatellite stability and therefore were classified as MMR proficient (MMR-P, 84.5%). 50/51 MMR-D tumors showed high frequency MSI and 50/51 demonstrated abnormal protein expression. In detail 39 tumors showed complete loss of MLH1 and PMS2 expression, 3 tumors loss of MSH2 and MSH6 expression, and 6 tumors selective loss of the MSH6 protein. A single carcinoma showed selective loss of PMS2 expression. The large majority (84.3%) of MMR-D tumors were localized in the proximal colon. MLH1 promoter methylation was observed in 34 MLH1/PMS2 negative carcinomas. On the basis of immunohistochemical data and MLH1 promoter methylation status, we can hypothesize that about 70% of MMR-D tumors were sporadic and 30% hereditary.

Conclusions: Our results indicate that immunohistochemistry is a rapid and suitable method for the identification of MMR-D CRCs. Pathologic screening of colorectal tumors should include analysis of expression of MLH1, MSH2 and MSH6 proteins, as the observed frequency of cases with selective loss of MSH6 expression was much higher than expected.

581 The Value of Prospective Analysis of Mismatch Repair by Immunohistochemistry in Colorectal Cancer in the Irish Setting

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Background: Hereditary Non-Polyposis Colorectal Cancer (HNPCC) accounts for 1-3% of all colorectal cancer (CRC) cases. Immunohistochemistry (IHC) for mismatch repair (MMR) proteins can aid in detecting these patients. Most CRC cases with deficient MMR (dMMR) status detected by IHC are sporadic and have a better prognosis than proficient MMR (pMMR) cases. Stratification of patients according to MMR status also assists in a more tailored approach to the use of adjuvant treatment. Since 2004, we have performed MMR IHC on all new CRC cases.

Design: Cases with IHC for MMR proteins MLH1, PMS2, MSH2 and MSH6 were identified from our database of 3130 patients. Clinical & pathological details were documented.

Results: IHC was performed on 752 cases. Median age at diagnosis was sixty-nine years. Loss of MMR protein expression was observed in 9%(n=67) of cases. There was loss of MLH1 & PMS2 in 81% (n=54), loss of MSH2 & MSH6 in 18% n=12) and exclusive loss of MSH6 in 1% (n=1).

Mismatch repair protein status and clinicopathological variables

	dMMR	pMMR	p value
Female	61%	43%	0.004
Family history CRC	32%	19%	0.013
Synchronous CRC	16%	7%	0.005
Metachronous CRC	13%	3%	<0.001
Right sided CRC	72%	29%	<0.001
Large tumour size (7-16 cm)	37%	14%	<0.001
Mucinous (>10%)	22%	9%	0.003
Poor differentiation	40%	13%	<0.001
Stage IV CRC	3%	18%	<0.001
Infiltrative tumour margin	39%	60%	0.001
Lymphovascular invasion	30%	49%	0.006

dMMR – deficient MMR protein, pMMR – proficient MMR protein

Specific HNPCC-associated mutations were identified in 8/67 cases (12%) of dMMR cases, 3/8 (37.5%) of whom were not suspected by Amsterdam criteria.

Conclusions: dMMR status was identified in 9% of CRC cases which is comparable to rates seen in other populations. dMMR cases were more likely to have a family history of CRC, synchronous & metachronous tumours & associated with larger tumour size, mucinous and poor differentiation. Adverse histological features such as lymphovascular invasion & infiltrative margin were less frequent in dMMR cases, with stage IV tumours in only 3% of cases. IHC for MMR identified eight HNPCC cases, focused the search for the specific mutation and led to appropriate screening and surveillance of patients and affected family members.

582 MUC Expression in Hyperplastic and Serrated Colonic Polyps: Lack of Specificity of MUC6

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Background: Previous studies have shown that hyperplastic (HP) and serrated polyps (SP) of the colon show variable degrees of gastric and intestinal-type MUC expression. One previously published study suggested that MUC6 expression, in particular, is specific for sessile serrated polyps (SSP) and, thus, can be used to distinguish these lesions from HP clinically (Owens, et al. Mod Pathol 2008;21:660-669). However, anecdotal data from our group suggests that MUC antibodies are not reliable in this differential diagnosis. Thus, the aim of this study was to systematically evaluate MUC expression in HP, SSP, and other SP and to determine the reliability of these antibodies in differentiating these polyp subgroups.

Design: Routinely processed specimens from 221 polyps (61 HP, 48 SSP, 51 SSP with dysplasia (SSP-D), 21 traditional serrated adenomas (TSA) and 40 conventional adenomas (CA)) were immunohistochemically stained with MUC1, MUC2, MUC5AC, and MUC6 and scored for extent (0-3), intensity (0-3) and crypt location (basal, superficial) of staining. The data was compared between the different polyp groups.

Results: MUC1, 2, and 5AC showed no significant differences in the location, extent, and intensity of staining between any of the polyp subgroups, including CAs. MUC6 expression was positive in 59%, 91%, 79%, 41%, and 43% of HP, SSP, SSP-D, TSA, and CA, respectively. Thus, MUC6 was not specific to SSP as reported previously. MUC6 staining was noted predominantly in the basal crypts. However, both the extent and intensity of MUC6 staining was significantly higher in SSP compared to HP (p value for extent: <0.001, intensity: 0.03). MUC6 expression was significantly decreased in TSA and CA compared to SSP either with or without dysplasia ($p<0.0001$ and $p<0.02$, respectively). For HP, no differences were noted in MUC6 expression between goblet cell, microvesicular and mucin depleted polyps.

Conclusions: Our MUC6 data supports the emerging theory that a subset of HP's represent a precursor to SSP. MUC6 is not specific for SSP and cannot be used reliably to distinguish this lesion from HP.

583 Sessile Serrated Polyps with Dysplasia: An Immunohistochemical Analysis of 51 Cases

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Background: Sessile serrated polyps (SSP), also termed sessile serrated adenomas, may develop dysplastic changes (SSP-D) believed to represent an intermediate step in the serrated pathway of carcinogenesis in the colon. Recent studies have suggested that some SSP-D show rapid progression to carcinoma. Dysplasia in SSP may show either traditional serrated adenoma (SA) or conventional tubular adenoma (CA) features. However, the biological properties of these two subtypes of SSP-D are unknown. The aim of this study was to evaluate the immunohistochemical properties of SSP-D, with particular emphasis on comparing those with SA versus CA-type dysplasia.

Design: 51 routinely processed SSP-D from 50 patients (mean age: 62 years, M/F ratio: 20/30, 30 right and 21 left colon) obtained via a 4 year pathology database search were evaluated for their location, type of dysplasia (SA or CA) and stained immunohistochemically for Ki67, p53 and beta catenin (for evaluation of their proliferative and molecular properties), MUC 1, 2, 5AC, and 6 (for their mucin differentiation profile) and AMACR (a neoplasia marker). Both the grade and intensity of staining was scored (0-3). Comparisons were made between the SSP-D polyps with SA-type dysplasia versus those with CA-type dysplasia.

Results: 23 SSP-D (45%) contained SA-type, 26 (51%) contained CA-type, and 2 (4%) contained mixed SA/CA-type dysplasia. The mean age of the patients and the size of the lesions were similar. However, a significantly higher proportion of SSP-D with CA-type dysplasia were found in males (54% vs. 26%, P=0.05) and a nearly significant increase in right and transverse colon location was noted as well (78% vs. 48%, p=0.06). Compared to SSP-D with SA-type dysplasia, SSP-D with CA-type dysplasia showed significantly higher aberrant surface Ki67 staining (100% vs 10%, $p<0.001$) and increased intensity of AMACR expression (strong staining in 83% vs. 0%, p=0.004). Cytoplasmic/nuclear beta catenin positivity was higher in SSP-D with CA-type dysplasia (92% vs. 57%), but not significantly (p=0.10). No differences between the polyp subgroups were noted with regard to MUC1, 2, 5AC, 6 or p53 staining.

Conclusions: SSP-D represent a morphologically heterogeneous group of neoplastic precursor polyps. Those with CA-type dysplasia are more common in males, more frequently found in the right and transverse colon, show higher proliferative rates, and more intense AMACR expression, suggesting that these polyps may have higher neoplastic potential. Further outcome studies of SSP-D with SA vs. CA-type dysplasia should be performed.

584 Anti-Phosphohistone H3 Shows Differential Expression in Barrett's Esophagus, Low-Grade Dysplasia, High-Grade Dysplasia, Adenocarcinoma Sequence

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Background: High degree of interobserver variability in grading of dysplasia in Barrett's esophagus (BE) demands a biomarker which can be utilized by the practicing pathologists. Phosphorylation of histone H3 (pHH3) occurs primarily during mitosis and has shown utility as a prognostic marker in meningioma, melanocytic tumors and vulvar intraepithelial neoplasia.This study evaluated the expression of pHH3 in the metaplasia-dysplasia-carcinoma sequence in BE, and determined its utility as a supportive marker in BE and associated dysplastic lesions.

Design: The study included 102 endoscopic biopsies from 46 patients [M/F ratio: 6.6, mean age: 62] with BE and different grades of dysplasia and adenocarcinoma (ACA). In all cases, H&E sections were reviewed by two gastrointestinal pathologists in a blinded manner, and the cases were only included after consensus diagnosis. 5µm sections from 28 biopsies with BE, 50 biopsies with low-grade dysplasia (LGD), 14 biopsies with high-grade dysplasia (HGD) and 10 biopsies of ACA were immunostained with anti-pHH3 antibody (rabbit polyclonal antibody , 1:400 dilution, Millipore, CA) after antigen retreival and endogenous peroxidase blocking. Anti-pHH3-labeled mitotic figures were counted in surface epithelium together with superfical and deep crypts per 10 consecutive high-power fields (using x40 HPF magnification) and the mean was calculated. Positive-staining nuclei that did not show chromatin aggregation were excluded.The mean anti-pHH3 positive cells were comapred between different lesions using student t test (SPSS, Chicago, IL). $p<0.05$ was considered significant.

Results: The result of immunohistochemical analysis for pHH3 is summerized in Table.

table

Lesion	Mean	SD	Range
BE (n=28)	0.65	0.49	0.00-1.80
LGD (n=50)	2.74	1.24	0.60-5.60
HGD (n=14)	6.44	2.48	3.10-10.00
EAC (n=10)	9.89	3.99	5.00-16.00

The difference in pHH3 staining between BE without dysplasia and those with LGD, HGD, or ACA was highly statistically significant ($p<0.0001$). The differences in pHH3 staining between LGD and HGD ($p=0.0001$) and between HGD and ACA ($p=0.002$) were statistically significant.

Conclusions: This study demonstrates that pHH3 expression is significantly increased in the neoplastic progression of BE. The immunostain for pHH3 can supplement morphologic assessment of BE and help differentiating the degree of dysplasia, particulaly in low grade dysplastic lesions.

585 Early Colorectal Perineurioma: A Frequently Missed Lesion?

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Background: Typical colonic perineuriomas are characterized by a stromal mucosal proliferation of monomorphic plump to spindle cells leading to obvious separation and disorganization of frequently serrated colonic crypts. In our experience however, a sizable amount of the cases show only a minimal degree of stromal proliferation and crypt separation. Accordingly, these perineuriomas can be misdiagnosed as hyperplastic polyps or mucosal tags. To bring attention to this previously undescribed morphological variant of colonic perineurioma (early perineurioma), we report the histological and immunohistochemical findings of a series of 11 cases.

Design: Eleven cases diagnosed as early perineuriomas were retrieved from the authors' files from among 49 cases of colonic perineuriomas. H&E stained sections were re-examined and immunostains for perineurial markers including epithelial membrane antigen (EMA), claudin-1, glut-1 and collagen type IV were performed. Clinical and endoscopic data were obtained from the patients' charts.

Results: Seven patients were male and 4 female (age range 44-71 yrs; median 62). All lesions were found during screening colonoscopy for colorectal carcinoma. Polyp size ranged from 2 to 4 mm. Eight (73%) were located in the sigma and in 5 (45%) cases there was an associated hyperplastic polyp. Histologically, early perineuriomas were characterized by small, non-contiguous nests or bundles of monomorphic plump or spindle stromal cells with eosinophilic cytoplasm and oval to fusiform nuclei, leading to minimal separation of straight, parallel crypts. In 8 (72%) cases, the crypts were serrated. In all cases, the foci of stromal proliferation expressed all 4 perineurial markers.

Conclusions: Early perineurioma is a morphologic variant of colonic perineurioma characterized by a limited proliferation of perineurial cells. This lesion can be misdiagnosed as hyperplastic polyp or mucosal tag. A panel of perineurial markers helps in reaching an accurate diagnosis.

586 Analysis of Microsatellite Instability, Protein Expression and Methylation Status of *hMLH1* and *hMSH2* in Gastric Carcinomas

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Background: Microsatellite instability (MSI) is a manifestation of defective DNA mismatch repair system. The majority of cancers of hereditary non-polyposis colorectal cancer (HNPCC) syndrome have MSI+ phenotype. The CpG island methylation has been recognized as an alternative mechanism of gene inactivation in the carcinogenesis. Hypermethylation of *hMLH1* mismatch repair gene has been revealed to lead the MSI in HNPCC. The colorectal cancers show distinctive clinicopathologic characteristics and prognosis according to the MSI status. However, there is a wide variety of results between MSI and clinicopathologic parameters in gastric carcinomas.

Design: To determine the correlation between MSI status and clinicopathologic characteristics in gastric carcinomas, five hundred twenty one gastric carcinomas by surgical resection were studied. We analyzed the correlation with clinicopathologic parameters, MSI status by using five microsatellite markers (BAT25, BAT26, D5S346, D2S123, and D17S250), expression of hMLH1 and hMSH2 protein by immunohistochemical stain, and methylation of *hMLH1* and *hMSH2* by methylation-specific polymerase chain reaction.

Results: In 521 cases, there were 50 (9.6%) high-frequency MSI (MSI-H) cases, 5 (1.0%) low-frequency MSI (MSI-L) cases, and 466 (89.4%) microsatellite stable (MSS) cases. In 50 MSI-H gastric carcinomas, the loss of hMLH1 and hMSH2 protein expression was 46 cases (92.0%) and 4 cases (8.0%), respectively. The MSI-H gastric carcinomas were significantly correlated with older age (≥50 years), expanding type of Ming's classification, lymphatic invasion, tumor multiplicity, and loss of hMLH1 protein expression ($P<0.05$). In MSI-H gastric carcinomas, the methylation frequency of *hMLH1* and *hMSH2* was 75.5% and 46.2%, respectively.

Conclusions: Our results suggest that epigenetic inactivation of *hMLH1* might play a role in the carcinogenesis of MSI-H gastric carcinomas. The immunohistochemical stain for hMLH1 protein expression could be used in routine diagnostic method for predicting MSI status.

587 Cancer Risk Stratification in UC Patients with Endoscopically Resected Dysplastic Polyps: Top-Down vs. Bottom-Up Dysplasia

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Background: Dysplastic polyps in the setting of UC are currently classified endoscopically as non-adenomalike, i.e., incompletely resectable, poorly circumscribed, irregularly-shaped or with stigmata of cancer, versus adenomalike, i.e., resectable, well-circumscribed and dome-shaped. Patients with non-adenomalike dysplastic polyps are at high risk of colorectal cancer (CRC) and are usually managed with immediate colectomy, whereas those with adenomalike polyps may be managed with polypectomy and endoscopic surveillance. Although these polyps overlap histologically, distinguishing histological criteria would be desirable, especially since endoscopists often find the endoscopic distinctions difficult to apply in practice. We carried out a cohort study comparing UC patients with endoscopically resected dysplastic polyps featuring superficial or top-down (TD) dysplasia and full-thickness or bottom-up (BU) dysplasia with respect to progression rates to CRC.

Design: Cohorts identified from an institutional database of UC patients undergoing surveillance examination in 1996-7 included patients with at least 1 dysplastic polyp and patients who were dysplasia-free (NoD) at their first colonoscopy at our institution. Each polypectomy slide was reviewed retrospectively and classified as having TD or BU dysplasia. Incompletely resected and poorly oriented polyps were excluded. Patients were followed until the development of colorectal cancer or censored at their last cancer-free examination. Differences in actuarial progression were examined by log-rank testing.

Results: Of 76 UC patients with dysplastic polyps (median follow-up 3.0y), 37 had at least one TD polyp and 39 had only BU polyps. 1 patient from the TD group and 5 from the BU group ultimately progressed to CRC. This difference was actuarially significant ($p=0.016$ by Log Rank testing). There was no difference in progression between patients in the TD and NoD groups (N=349, median follow-up 7.0y), 18 of whom developed CRC ($p=0.86$).

Conclusions: The top-down pattern of polypoid dysplasia in UC is a histological marker of low risk for CRC development, permitting conservative management with polypectomy and continued surveillance. The bottom up pattern may encompass lesions of low and high risk, and requires evaluation based on the current endoscopic criteria.

588 Enterocolic Lymphocytic Phlebitis Is a Common Accompaniment of Diversion Colitis with Coexistent Inflammatory Bowel Disease

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Background: Enterocolic lymphocytic phlebitis (ELP) is a rare entity characterized by circumferential, predominantly lymphocytic infiltration of small submucosal and/or subserosal veins with sparing of arteries. Thought to be a hypersensitivity reaction, its etiopathogenesis and associated lesions are yet to be well elucidated. The aim is to explore the association and incidence of ELP in diversion colitis with and without inflammatory bowel disease.

Design: H&E sections of 150 colon resection specimens (right, left and subtotal) consisting of the following were examined: 26 cases of diversion with ulcerative colitis, 26 cases of ulcerative colitis without diversion colitis, 3 cases of diversion with Crohn's disease, 19 cases of Crohn's disease without diversion colitis, 6 cases of diversion colitis without inflammatory bowel disease and 70 random resection cases with other pathologic findings (carcinoma, adenoma, ischemia, diverticular disease). ELP was defined/diagnosed as submucosal veins showing a concentric, perivascular dense lymphocytic infiltrate with swelling of endothelial cells, cytoplasm of some showing vacuolization. A case was regarded as showing ELP if at least one vein fulfilled the histologic criteria listed above.

Results: ELP was identified in 18/26 cases of diversion colitis with ulcerative colitis, all 3 cases of diversion colitis with Crohn's disease, 2/19 cases of Crohn's disease without diversion colitis and 1 of 26 cases of ulcerative colitis without diversion, 1/6 cases of diversion colitis (a case of diverticular disease) without associated inflammatory bowel disease and in 5/70 cases lacking diversion colitis, ulcerative colitis or Crohn's disease (the so-called random group). One of these 5 cases was for resection of a large tubulo-villous adenoma showing lymphocytic colitis and ELP in a patient on multi-drug therapy.

Conclusions: This series shows that ELP occurs preferentially in colons showing features of diversion colitis with ulcerative colitis or Crohn's disease. It is extremely uncommon in the colons harboring any of these 3 conditions alone. We speculate that a

synergistic effect of altered bowel contents from diversion together with the pre-existing immunologic triggers that occur in inflammatory bowel disease, are possible reasons for the occurrence of ELP in this unique set of conditions.

589 Serrated Colon Polyps Are Not Diagnosed with a High Degree of Consistency in an Average Community Practice Setting: An Inter-Observer Agreement QA Study

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Background: Sessile serrated adenoma(SSA) is a putative precursor of colorectal carcinoma and requires different clinical management than conventional hyperplastic polyp(HPP). While published studies show diagnostic agreement at Kappa levels of 0.41 - 0.83, it is our impression that there is significantly lower agreement in general practice due to inconsistent use of diagnostic criteria and terminology for SSA.

Design: We searched our 2007-06 database for 50 consecutive cases of HPP, SSA, and serrated adenoma(SA), and 35 cases of serrated polyp(SP). 50 normals were also included. Exclusion criteria were IBD and colonic adenoma/adenocarcinoma. Biopsy site, largest fragment size and age/gender of patient were noted. Three GI pathologists and a resident each did a blinded review of the 235 cases and assigned them to: normal, HPP, SSA, traditional serrated adenoma(TSA), mixed polyp(MX), adenoma(TA/VA) or other. A consensus diagnosis was achieved at multiheader microscopic review. For the study it was assumed that a diagnosis of SP equated to SSA, and SA to TSA. Unweighted Kappa statistics were used to compare levels of agreement.

Results: In 176 cases (original normals and others excluded), there was agreement between the original and consensus diagnosis in 95 cases (54%). There was 70% agreement for SSA and 52% for HPP. The SP cases were evenly split between HPP and SSA (46% each). The Kappa value for agreement between the four reviewers was 0.401(moderate); and between the original and consensus diagnosis was 0.352 (fair). The Kappa values for each reviewer were: 0.391, 0.672, 0.612, and 0.643. The diagnostic sensitivity for clinically significant lesions versus conventional HPP was 85%, and specificity was 44%.

	HPP	SSA	TSA	MX	TA/VA
HPP (original dx)	26	13	0	0	3
SSA (original dx)	9	35	5	1	0
SP (original dx)	16	16	0	1	0
SA (original dx)	13	10	7	1	14

Conclusions: A significant number of SSAs (18%) are undercalled as HPPs, and a large number of HPPs (58%) are overcalled as SSA/SP/SAs, resulting in potential inappropriate patient management. Even with the use of defined morphologic criteria, agreement is only moderate to good among reviewers. There is an urgent need for more accurate diagnostic modalities for SSA and prospective data about its clinical significance.

590 Hilar Cholangiocarcinoma: A Comparison of Endoscopic Transpapillary Brush Cytology an Forceps Biopsy

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Background: Analysis of current epidemiological data shows an increasing incidence of extrahepatic cholangiocarcinoma . The prognosis of this neoplasm is poor, mainly due to the late appearance of symptoms and difficulties in separating benign and malignant strictures of bile ducts both by imaging, as well as, in endoscopic biopsy material. An increase in the diagnostic sensitivity and specificity is therefore urgently needed.

Design: All cases with histologically or cytologically proven extrahepatic cholangiocarcinoma with available clinical follow-up from the period 1995 to 2005, in whom both transpapillary brush cytology and forceps biopsy had been performed for diagnostic purposes, were included in the study. Patients with intrahepatic cholangiocarcinoma, and patients without surgery, in whom no evidence of disease progression during a period of 18 months made a malignant diagnosis unlikely, were excluded. The clinical and follow-up data, as well as, all available diagnostic material was reviewed.

Results: Fifty-eight patients with a median age of 68 years with malignant bile duct tumours staged according to Bismuth were included in the study. In 18.9% of patients, more than one endoscopy with diagnostic biopsy had been performed. The mean number of tissue sampling sessions was 1.3 per patient. Brush cytology alone had a sensitivity of 41.4% (24/58), compared to 53.4% (31/58) for forceps biopsy. Combination of cytology and biopsy resulted in a minor increase of sensitivity to 60.3% (35/58). 34.4% (20/58) patients had both positive cytology and histology, 19% (11/58) had positive histology only, and 6.9% (4/58) were positive only in cytology.

Conclusions: The combination of forceps biopsy and brush cytology for the endoscopic diagnosis of proximal cholangiocarcinomas according to Bismuth classification leads to a moderate increase in diagnostic sensitivity, but still fails to establish the diagnosis in almost 40% of cases. Since timely diagnosis is of importance for potentially curative surgery, introduction of supplementary techniques such as fluorescence in situ hybridization should be evaluated for cases of morphologically negative specimens of bile duct strictures.

591 Ectopic Expression of Gastric Mucin MUC6 in Colorectal Adenomas and Carcinomas Is Associated with Microsatellite Unstable Pathway

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Background: Microsatellite unstable (MSI) colorectal cancer (CRC) has better prognosis and different response to chemotherapy than microsatellite stable (MSS) colon cancer. The distinction between MSI and MSS CRC is important but the molecular tests are expensive and time-consuming. Sporadic MSI CRC commonly arises from sessile serrated adenoma (SSA) while its hereditary form, herediatary non-polyposis colorectal cancer (HNPCC) develops in adenomatous polyp (AP). Our previous study showed that MUC6 was expressed in SSA and subsequent dysplasia (Modern Pathology 2008;21:660-9). We now test the hypothesis that MUC6 is a potential marker for MSI CRC and precursor lesions.

Design: Twenty-five MSI-high CRCs (15 sporadic, 10 HNPCC), 20 MSS CRCs (17 sporadic, 3 familial adenomatous polyposis (FAP)), and 35 APs (20 MSS-sporadic, 10 MSS-FAP, and 5 MSI-HNPCC) were reviewed. A clinical diagnosis of HNPCC is based on modified Bethesda criteria. FAP is diagnosed based on germ-line mutation of APC gene. The microsatellite status was confirmed by a panel of 5 NCI markers or an immunohistochemical panel of MLH1, MSH2, and MSH6. Formalin-fixed paraffin-embedded sections were stained with an antibody to MUC6.

Results: Of the 25 MSI CRCs, 14 of 15 (93%) sporadic subtype and 8 of 10 (80%) HNPCC subtypes were positive for MUC6. Four of 5 (80%) HNPCC APs were positive for MUC6. The MUC6 positivity has no correlation with mucinous differentiation. Either mucinous or non-mucinous components may be MUC6(+). The extent and intensity of MUC6 staining tends to decrease with poorer grade of differentiation or higher grade of dysplasia. None of 20 MSS CRCs and 30 MSS APs, either sporadic or APC subtype, were positive for MUC6.

MUC6 in colorectal cancers and adenomatous polyps

Diagnosis	Microsatellite Status	Case No.	MUC6(+)	Percent
Sporadic CRC	MSI	15	14 *	93%
Sporadic CRC	MSS	17	0 *	0%
Hereditary CRC, HNPCC	MSI	10	8 ¶	80%
Hereditary CRC, FAP	MSS	3	0 ¶	0%
AP, HNPCC	MSI	5	4 §	80%
AP, sporadic or FAP	MSS	30	0 §	0%

*p= <0.0001(Chi-Square);¶p=0.035;§p=<0.0001(Fisher's exact test)

Conclusions: Ectopic expression of a gastric mucin MUC6 in colorectal cancers and adenomatous polyps, both sporadic and hereditary forms, is highly associated with microsatellite unstable pathway. MUC6 may be a useful marker for the screening of the microsatellite status before the expensive molecular study. MUC6 or its related DNA loci may be susceptible targets for mismatch repair mechanism.

592 Loss of Heterozygosity at 18q21 and Increased Proliferative Index Are Associated with Appendiceal Mucinous Carcinomatosis

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Background: Disseminated appendiceal mucinous neoplasms are categorized as low grade appendiceal mucinous neoplasia (LAMN) and high grade appendiceal mucinous carcinoma (AMC), a classification which is associated with prognosis. Few studies to date have examined genetic or immunophenotypic differences between low grade and high grade disseminated appendiceal neoplasms.

Design: 30 consecutive cases of disseminated primary appendiceal mucinous neoplasms from 1/2007- 7/2008 were reviewed and 27 cases could be classified as LAMN or AMC. Three cases regarded as intermediate were excluded from analysis. The cases were evaluated for KRAS mutation in codons 12 and 13; loss of heterozygosity (LOH) at 16 microsatellite markers on chromosomes 1p36, 3p25, 5q23, 7q31, 7q22, 9p21, 10q23, 17p13, 18q21; and expression of p53, p16, Smad4 and Ki-67 by immunohistochemistry (IHC).

Results: 13/27 cases were classified as AMC. Oncogenic KRAS mutations were found in 10/13 AMC and 10/14 LAMN. Results of LOH analysis at selected loci are shown below; other loci showed no association with tumor grade.

LOH in LAMN and AMC

	18q21 LOH (D18S487)	10q23 LOH (D10S1173)	17p13 LOH (D17S1289)
LAMN	0/9	0/12	1/8
AMC	5/11 *	3/11 §	4/8 ¶

*p=0.038, §p=0.09,¶p=0.28(Fisher's exact)

Ki-67 proliferative index >10% was seen in 12/12 AMC and 4/11 LAMN (p=0.001, Fisher's exact). Strong nuclear p53 expression was seen in 3/12 AMC and 0/11 LAMN. Smad4 and p16 expression by IHC showed no correlation with tumor grade, LOH at 18q21 or LOH at 9p21. However, there was abnormal p53 nuclear expression in 4/4 cases with LOH at D17S1289 on 17p13. Interestingly, LOH at 18q21 and 10q23 exclusively occurred in AMC with KRAS mutations. Loss of Smad4 protein expression was also more common in appendiceal neoplasms with KRAS mutations.

Conclusions: In this series, elevated Ki-67 proliferative index, LOH at 18q21 and 10q23 are associated with high tumor grade (AMC). Oncogenic KRAS mutations are frequently found in appendiceal mucinous neoplasms, including localized appendiceal adenomas, indicating that KRAS mutation may be a tumor initiating event. Given that LOH at 18q21 and 10q23 were found exclusively in appendiceal carcinomas with KRAS mutations, LOH at these loci may be associated with genetic progression to carcinoma. Additional studies to evaluate mutations in candidate genes and confirmation of copy number changes involving these chromosomal loci is required.

593 Expression of Anti-Apoptotic Mcl-1 Protein Correlates with Higher Grade and Stage and Is a Marker of Metastasis in Colorectal Cancer

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Background: Mcl-1 (myeloid cell leukemia-1) plays a critical role in inhibiting apoptosis in well-differentiated cells by sequestering BAD, BID, and BAX and other apoptotic molecules. pAKT (phosphorylated serine-threonine protein kinase) is known to block apoptosis by facilitating the interaction of BAD with BCL-XL . Expression of pAKT and Mcl-1 have been described in colon cancer, however, the relationship between pAKT and Mcl-1 has not yet been reported.

Design: Immunohistochemistry was performed using stage oriented cancer tissue microarray (TMA) possessing 101 colorectal adenocarcinomas (CRC) of different stage, 8 adenomas (AD), and 14 normal colorectal mucosa (NR) samples that were analyzed using mouse monoclonal antibody toward pAKT (clone 587F11, 1:100 dilution, Cell Signaling Tech.) and Mcl-1 (clone RC13, 1:400 dilution, GeneTex Inc.). Mcl-1 and pAKT score, stage, and grade were compared using Spearman's correlation coefficient. Metastasis and no metastasis groups were compared using the Wilcoxon rank sum test.
Results: The Mcl-1 and pAKT scores were compared for the cohorts. The mean (SD) pAKT expression in NR (14) was 2.0 (1.4), in AD (8) was 3.0 (1.7), and in CRC (101) was 5.6 (2.4). These differences were statistically significant. For Mcl-1 the mean (SD) expression was 4.1 (1.7) in NR, 3.2 (1.2) in AD, and 3.3 (2.6) in CRC. There was correlation between Mcl-1 and pAKT expression during various stages of colon carcinogenesis ($p = 0.04$). Mcl-1 showed direct correlation with tumor grade ($p = 0.001$), tumor stage ($p = 0.02$) and with the presence of metastasis ($p = 0.008$).
Conclusions: We found that Mcl-1 correlates with tumor grade and stage and it is a marker for metastatic potential. The association between Mcl-1 and pAKT expression reflects the common anti-apoptotic function of these proteins. We also report the up regulation of pAKT during the transition from normal colorectal mucosa to carcinoma.

594 Utilization of Peripherin and S-100 Immunostaining in the Diagnosis of Hirschprung's Disease

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Background: Evaluation of rectal biopsies for ganglion cells is performed for patients suspected to have Hirschprung's Disease. At times, however, identification of ganglion cells is difficult, especially in newborns. As an established adjunct, frozen tissue can be obtained at the time of biopsy for acetylcholinestease histochemical staining. As an alternative we developed a protocol using peripherin and S-100 immunostaining as an adjunct to H and E identification of ganglion cells.
Design: 112 cases of rectal biopsy for evaluation of Hirschprung's Disease were compiled from the archives of the Medical College of Georgia from 2002-2008. Initial evaluation consisted of eight levels of H and E staining and two levels each for immunostaining with peripherin and S-100 (levels 3 and 8). If on initial evaluation ganglion cells are not identified, then serial H and E sections and additional peripherin stains were performed. Follow-up to confirm stooling was obtained on 50% of patients with proven ganglion cells and on all patients with aganglionic segments.
Results: Peripherin immunostaining was unequivocally identified in the cytoplasm of ganglion cells at all ages. Of the 112 patients, 85 had ganglion cells. Of these, 79 cases were diagnosed on the first set of levels (93%), resulting in a less than 24 hour turn around time. Six cases required the full protocol to identify ganglion cells. One case was initially negative, but had ganglion cells on repeat biopsy. The initial biopsy had low columnar mucosa and was felt to represent the physiologic aganglionic segment. Twenty-five cases were devoid of ganglion cells. Two cases had repeat biopsies, both negative for ganglion cells. 79% of these cases demonstrated submucosal neural hypertrophy on S-100 staining. Twenty-four patients had confirmed aganglionic segments at the time of colonic resection. One patient had OR evaluation confirming aganglionosis, with colostomy only (multiple congenital defects). All 24 of the Duhamel resection patients stooled normally post-op.
Conclusions: In this study, the use peripherin and S-100 immunostaining as an adjunct to H and E staining of rectal biopsies for Hirschprung's disease was significantly sensitive and specific for the identification of ganglion cells. Of importance, the diagnosis of the majority of patients with ganglion cells could be rendered within 24 hours without the need for collecting frozen tissue. In addition, this protocol appears to accurately diagnose patients with Hirschprung's disease.

595 Adenocarcinoma of the Small Intestine: A Multi-Institutional Study of 197 Cases

S-M Hong, E Yu, JH Kim, YK Bae, K-T Jang, H-K Chang, ES Jung, G-S Yoon, H-I Bae, JM Kim, Y-H Oh, GI Kim, SJ Jung, MJ Gu, JY Kim, KY Jang, S-Y Jun, DW Eom, KW Kwon, GH Kang, JB Park, SW Hong, JS Lee. Johns Hopkins Medical Institutions, Baltimore, MD; Korean Small Intestinal Cancer Study Group, Seoul, Korea.
Background: Small intestinal adenocarcinoma (SIAC) is a rare malignant neoplasm and its clinicopathologic characteristics have not been systemically evaluated.
Design: A total of 197 SIAC cases were collected from 22 institutions in Korea, and were evaluated for clinicopathologic factors that affect prognosis of SIAC patients using univariate and multivariate analyses.
Results: Mean patient age was 59 and male to female ratio was 1.7:1. Tumors were located in the duodenum of 108 cases (54%), the jejunum in 56 (28%), the ileum in 29 (15%), and an unspecified site in 4 (3%). Predisposing conditions were observed in 23 cases (12%), including 17 cases with sporadic adenomas, 3 with Peutz-Jeghers syndrome, 2 with Meckel's diverticulum, and 1 with Crohn's disease. Synchronous or metachronous malignant tumors were identified in 31 cases (16%), including 13 colorectal and 10 stomach cancers. About 90% of tumors were either pT3 (108 cases) or pT4 (66 cases) classification. Median survival time for entire SIAC patients was 38.5 months. SIAC patients with accompanying adenomas tended to have a tumor with well-differentiation ($P<0.001$), a more polypoid growth pattern ($P<0.001$), a lower pT classification ($P<0.001$), less perineural ($P=0.01$) and lymphatic ($P=0.04$) invasion than those without adenomas. SIAC patients with adenomas (76.9%) had a significantly better 5-year survival rate than those without adenomas (37.2%, $P=0.002$). pT ($P<0.001$) and pN ($P=0.001$) classifications, location of tumor ($P=0.001$), vascular invasion ($P=0.01$), and radiation therapy ($P=0.01$) had significantly different patient survival by univariate analysis. By multivariate analysis, only pT ($P=0.01$) and pN ($P=0.03$) classifications remained significant prognostic factors.
Conclusions: 1) SIACs are diagnosed in advanced stage, therefore development of strategies for detection in earlier stages is required. 2) SIAC patients with adenomas have a better survival than those without adenomas. 3) Like other gastrointestinal tract tumors, pT and pN classifications are the most important prognostic factors.

596 Simplified Staging for Extrahepatic Bile Duct Carcinoma

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Background: The current T classification of AJCC staging system may not provide meaningful prognostic information in patients with extrahepatic bile duct (EBD) carcinoma. The purpose of the current study was to evaluate the current AJCC staging of EBD carcinoma and to assess whether depth of invasion better predicts outcome following resection of EBD carcinoma.
Design: Clinicopathologic data on patients undergoing resection of EBD carcinoma were collected and analyzed. Specifically, the depth of EBD carcinoma invasion from the basal lamina of the adjacent normal epithelium to the most deeply-advanced tumor cells was measured. Overall survival was assessed using the current T classification from AJCC staging system as well as depth of tumor invasion. Analyses were performed using univariate and multivariate analyses.
Results: There were 234 patients with EBD carcinomas available for review. Depth of invasion was classified into 3 groups: group 1 (<5 mm, 44 cases), group 2 (5-12 mm, 117 cases) and group 3 (>12 mm, 73 cases). The current AJCC T classification was unable to accurately stratify patients with regard to prognosis (median survival: T1, 40.8 months; T2, 19.9 months; T3, 17.8 months; T4 20.2 months; $P=0.15$). In contrast, depth of EBD carcinoma invasion was able to stratify patients with regards to prognosis (median survival: groups 1, 40.9 months; group 2, 23.5 months; group 3 14.0 months; $P<0.0001$). On multivariate analysis, invasion depth (5-12 mm of invasion depth, $P=0.04$; >12 mm of invasion depth, $P=0.0002$), perineural ($P=0.03$), and vascular invasion ($P=0.02$) were independent predictors of patient survival.
Conclusions: Measurement of invasion depth can discriminate prognosis of patients with EBD carcinoma better than the current AJCC T classification. As such, future AJCC T classification schemas for EBD carcinoma should include measurement of tumor invasion depth.

597 KRAS Analysis for EGFR-Directed Therapy in Colorectal Cancer (CRC): A Review of Greater Than 2500 Cases Using an Allele-Specific Assay for Detection of 12 Mutations

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Background: EGFR promotes tumor growth in part through KRAS stimulation. Inhibition of EGFR is a frequent therapy in CRC. However, when KRAS undergoes mutation, KRAS drives tumor growth independent of EGFR and EGFR-directed therapy. To exclude ineffective EGFR-directed CRC therapy, KRAS mutation analysis has recently emerged as a significant CRC biomarker. The purpose of this study is to review KRAS mutation results of CRC samples received for testing in our laboratory.
Design: KRAS mutation analysis was performed on tumors from 2583 colorectal cancer patients. Biopsy material was enriched for tumor using manual microdissection. The extracted DNA was quantified and amplified by polymerase chain reaction (PCR) using primers to exon 2 of the KRAS gene. PCR products were subjected to single nucleotide extension to detect mutation at codons 12 and 13; primer extension products were analyzed by capillary electrophoresis.
Results: Eleven of 12 possible mutations in codons 12 and 13 were detected. The overall mutation detection rate was 40%. 78% of the mutations were present in codon 12 with the remaining 22% in codon 13. Within codon 13, the vast majority of mutations (93%) were G13D , with 5% G13C, 1% G13V, and 1% G13R. The latter 3 mutations are rarely examined in most current KRAS analyses.
Conclusions: The detection rate for KRAS mutations in CRC samples tested in our laboratory is consistent with published data. Most laboratories currently performing KRAS testing examine only 7 mutations. In our laboratory 11/12 mutations in codons 12 and 13 were detected. While the frequency of some of these mutations is low, a test that maximizes the detection rate is needed since critical therapeutic decisions are made based on KRAS mutation status.

598 Gastroesophageal Junctional Carcinomas in Chinese Patients Show Distinct Clinicopathologic Features

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Background: The pathogenesis of carcinomas involving the gastroesophageal junction (GEJ) is poorly understood. In the United States (US), most authorities believe that GEJ carcinomas are related to Barrett esophagus (BE). However, in China, BE is infrequent but GEJ carcinomas are common. The aim of this study was to investigate and compare the clinicopathologic features of GEJ carcinomas that developed in patients in the US (Caucasians) versus China.
Design: Routinely processed tissue slides of untreated GEJ carcinoma resection specimens were retrieved from 29 US and 35 Chinese patients. Clinical information and histological features of GEJ carcinomas were reviewed and compared between the two patient groups. GEJ carcinoma was defined as a tumor with its epicenter within 2.5 cm of the GEJ.
Results: Compared to US patients, GEJ carcinomas in Chinese patients occurred at a younger age (63 vs 68 years; $p<0.05$) and were larger in size (mean size: 5 vs 3cm; $p<0.01$). All GEJ tumors were adenocarcinomas (100%) in US patients, but tumors in Chinese patients were adenocarcinoma in 60%, acinar cell carcinoma in 9%,

adenosquamous carcinoma in 18%, and colloid-signet ring cell carcinoma in 11%. BE was identified in 92% of US patients, but only in 7% of Chinese cases ($p<0.0001$). Furthermore, adjacent gastric mucosa showed intestinal metaplasia and dysplasia in 51% and 43% of Chinese patients, respectively, which were significantly higher values than those found in US patients (10% and 3%, respectively) ($p<0.0001$).

Conclusions: GEJ carcinomas that develop in Chinese patients are clinically and pathologically different from those in US patients. These data may indicate different pathogenetic mechanisms for GEJ carcinomas in China compared to the US.

599 Expression Pattern of LGR5, a Recently Described Intestinal Stem Cell Marker, in Human Duodenum and Duodenal Adenomas

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Background: No well-defined marker of human intestinal stem cells has been described. Previously suggested stem cell markers have been too broadly expressed to be specific and the role of stem cells in intestinal neoplasia remains to be determined. LGR5, an orphan G-protein coupled receptor related to glycoprotein hormone ligands such as TSH, FSH and LH receptors, has been identified as a Wnt target gene with restricted crypt expression. In murine studies, the LGR5+ crypt based columnar cells (CBCs) show features unique to stem cells: label-retention, pluripotency, and the capacity to maintain epithelial renewal over extended periods of time. We aim to evaluate expression of LGR5 in human small intestine and contrast this with its expression in adenomatous proliferations of the duodenum.

Design: 10 cases of duodenal tubular adenomas and 7 controls (unremarkable duodenum, chronic duodenitis) were analyzed by LGR5 immunohistochemistry. The specificity of the antibody (Abcam, Cambridge, MA) was confirmed by blocking it with the immunogenic peptide. We strictly defined LGR5+ CBCs as cells with crisp membranous and finely granular cytoplasmic staining. We analyzed LGR5+ non-adenomatous versus adenomatous transversally sectioned glands comparing the ratio of positive cells to the total number of cells per gland between the two groups. Statistical significance was assessed with student T-test.

Results: Duodenum stains positive for LGR5+ CBCs with a particular morphology: slender pyramidal cells with large nucleus and few delicate apical projections, located mostly, but not exclusively, in the crypt base. In normal duodenum, there are 1-2 LGR5+ CBCs in a positive gland (5 LGR5+ CBCs per 100 cells). 6/10 duodenal tubular adenomas showed areas with an absolute increased number of LGR5+ cells (12 LGR5+ CBCs in 100 cells), averaging 10 CBCs per positive adenomatous gland. This difference between non-adenomatous and adenomatous glands was significant ($p<0.05$). Four adenomas showed no staining.

Conclusions: Based on previous mouse studies, LGR5 immunostaining in normal human duodenum confirms the presence of intestinal stem cells and highlights their morphologic characteristics. The increased absolute number of LGR5+ cells in positive adenomatous glands suggests that dysregulation of stem cell activity may be involved in duodenal adenoma pathogenesis. The lack of staining in some adenomas could be explained by sampling or may be indicative of non-stem cell pathways for adenoma development.

600 Loss of Nuclear PTEN Expression Is a Marker of Poor Prognosis in Stage II Colorectal Adenocarcinoma

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Background: Tumor suppressor *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) is an important negative regulator for PIP3/Akt signaling pathway that promotes cell proliferation and inhibits apoptosis. Inactivation of *PTEN* by mutation, deletion and promoter hypermethylation has been demonstrated in a range of cancers. We investigated whether loss of nuclear PTEN protein expression correlated with conventional clinicopathologic parameters and patient's survival.

Design: Immunohistochemical staining for PTEN was performed on the tissue microarray of 19 normal colonic mucosa, 14 adenomatous polyps, 482 adenocarcinomas and 56 metastatic lymph nodes. Data was analyzed by Chi-square test, Cox regression harzard model and log-rank test with Kaplan-Meier curves.

Results: All 19 normal colonic mucosa (100%) were positive for PTEN and 12 (85.7%) of 14 adenomatous polyps were positive for PTEN. However, 241 (50.0%) of 482 colorectal adenocarcinomas were positive for PTEN. Moreover, 26 (46.4 %) of 56 metastatic lymph nodes were positive for PTEN. No correlations were found between PTEN expression and age, gender, tumor size, depth of invasion, LN metastasis, distant metastasis, AJCC stage, degree of differentiation, and lymphovascular invasion. In survival analyses, patients with PTEN-negative adenocarcinoma revealed a poor overall and disease free survival ($p = 0.030$ and $p = 0.046$, respectively). The overall survival and disease free survival were significantly poor especially in PTEN-negative stage II adenocarcinoma ($p = 0.011$ and $p = 0.025$, respectively).

Conclusions: The nuclear PTEN expression was gradually decreased during normal-adenoma-adenocarcinoma-metastasis sequence, suggesting the important role of PTEN on carcinogenesis. Moreover, loss of PTEN expression was a marker of poor clinical outcome in colorectal cancer patients.

601 Survival by Depth of Tumor Invasion in PT1 Esophageal Adenocarcinoma: An Analysis of 182 Patients

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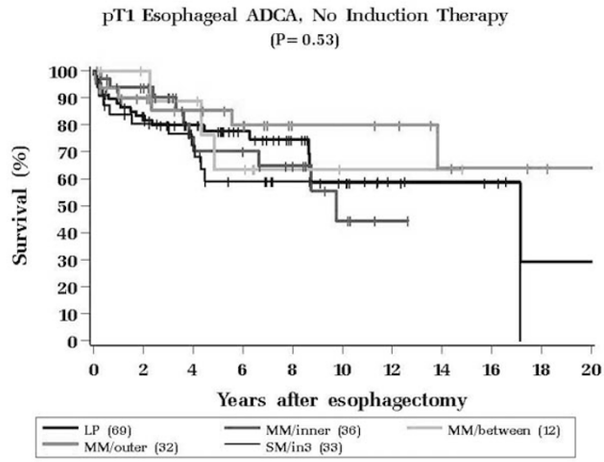
Background: Duplication of the muscularis mucosa (MM) is a known finding in Barrett's esophagus. Few studies have evaluated the relationship between depth of invasion in intramucosal carcinoma (IMC) and survival in a large group of pts.

Design: 182 pts with pT1 esophageal adenocarcinoma treated by esophagectomy (no induction therapy) were identified. IMC was subdivided into invasion into lamina propria (LP), inner muscularis (IMM), between inner and outer MM (BMM) and outer muscularis (OMM) and compared to tumors invading the inner third of the submucosa (SM-I). Gender and pathologic N-stage were compared among the five groups using Chi-square test. Age and yrs follow-up were compared among groups using the Kruskal-Wallis test. Survival was estimated using the Kaplan-Meier method and compared among the five groups using the log-rank test.

Results: For IMC, depth of invasion was LP (n=69), IMM (n=36), BMM (n=12), OMM (n=32). 33 pts had SM-I. Pt characteristics are summarized in Table 1. Length of survival by group is summarized in Figure 1. There was no significant difference in gender or age among groups. 3/149 pts with IMC had N1 disease (2%; LP-2, BMM-1). 2/33 pts with SM-I had N1 disease (6%).

Patient Characteristics by Depth of Invasion

Group (N)	Male	Mean Age±SD (R)	pN0	pN1	Mean yrs follow up±SD (R)
All pts (182)	156 (85.7%)	63±10 (35-81)	177 (97.3%)	5 (2.7%)	5.9±4.7 (0.1-20.5)
LP (69)	56 (81.2%)	62±10 (42-79)	67 (97.1%)	2 (2.9%)	6.0±4.3 (0.1-20.5)
IMM (36)	32 (88.9%)	64±11 (35-79)	36 (100%)	0	4.9±4.0 (0.2-12.6)
BMM (12)	10 (83.3%)	61±10 (48-74)	11 (91.7%)	1 (8.3%)	5.6±4.7 (0.2-14.8)
OMM (32)	30 (93.8%)	63±11 (36-80)	32 (100%)	0	6.8±6.1 (0.1-20.4)
SM-I (33)	25 (84.8%)	66±9 (48-81)	31 (93.9%)	2 (6.1%)	6.1±4.6 (0.1-16.6)
P-value	0.52	0.51	0.32	0.32	0.85



Conclusions: Depth of invasion within IMC does not impact survival. SM-I pts had similar survival to those with IMC-OMM.

602 High-Level Expression of HSP90 Is an Independent Prognostic Factor Predicting Recurrence in Intermediate and High-Risk Gastrointestinal Stromal Tumor

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Background: Despite the therapeutic success of imatinib in gastrointestinal stromal tumor (GIST), patients often develop resistances to the kinase inhibitor, generally resulted from secondary mutations. It is, therefore, important to identify alternative signaling targets for therapies and relevant prognostic factors. The mutant kinase proteins can be targeted using heat shock protein 90 (HSP90) inhibitor, which result in degradation of activated KIT and/or PDGFRA, or using KIT transcriptional repressors.

Design: To evaluate HSP90 expression as a prognostic marker and therapeutic target in GISTs, intermediate- and high-risk of aggressive behavior GISTs in a single institute between 1998 and 2005 were analyzed immunohistochemically. Clinicopathologic features and mutation results of KIT and PDFGRA genes were compared with clinical outcome in 57 patients followed up after complete surgical resection of GIST.

Results: HSP90 was expressed in 100% of cases and the intensity was strong in 9, moderate in 27, and weak in 21 cases. Forty eight (84.2%) GISTs had KIT mutations; 43 with exon 11 mutations (25 deletion, 5 duplication and 13 missense) and 5 with exon 9 duplication mutations. After a median follow-up of 64.1 months, 24 cases (42.1%) showed recurrence of disease and the recurrence-free survival rate was 87%, 58% and 51% at 1, 3 and 5 years, respectively. On univariate analysis, histologic subtype, risk grade, mitotic rate, intensity of HSP90 expression and specific KIT mutation types had prognostic importance ($p<0.05$). Patients with exon 11 deletion mutations or deletions affecting codon 557 or 558 had higher rates of recurrence. In multivariate analysis by Cox proportional hazards model, high risk ($p=0.02$, hazard ratio 7.087) and strong HSP90 immunoreactivity ($p=0.02$, hazard ratio 8.8) have been identified as independent prognostic variables for recurrence-free survival.

Conclusions: These findings highlight that HSP90 is an independent prognostic factor predicting recurrence and moreover, warrants clinical evaluation as potential therapeutic targets in patients with intermediate or high-risk GIST.

603 The Variants of Gastric Epithelial Dysplasia Display Different Biologic Characteristics: An Immunohistochemical Study of p53, Ki-67, beta Catenin and Smad4 Expression

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Background: Gastric epithelial dysplasia (GED) can be morphologically categorized into 2 types:adenomatous (or intestinal) and foveolar (or gastric / type II). While no distinct genetic differences have been demonstrated between these subtypes thus far, the immunophenotypic expression of p53, Ki-67, beta-catenin and Smad4 has not been simultaneously evaluated in this context previously.

Design: 58 cases of GED were immunohistochemically evaluated for expression of p53, Ki-67, beta-catenin and Smad4 and the findings were correlated with the morphologic subtypes. Accordingly, GED was classified as adenomatous, foveolar, or hybrid (showing features of both types), based on routine histologic evaluation.

Results: An adenomatous morphology was diagnosed in 26/58 cases (44.8%), hybrid in 18/58 cases (31.0%) and a foveolar subtype was observed in 14/58 cases (24.2%). The expression of the biologic immunomarkers was different between both dysplastic variants: the foveolar and hybrid types when analyzed as a group showed more p53 expression (p=0.012) and higher Ki-67 proliferation index (p=0.006) when compared to the adenomatous type. Conversely, nuclear beta-catenin expression was more common in adenomatous type (10/16, 62.5%) than foveolar (2/14, 14.3%), hybrid type (5/18, 27.7%), but it was statistically insignificant (p=0.168). There was no difference of Smad4 expression among three types of GED.

Conclusions: The various expression of p53 and Ki-67 suggests biologic differences between the in three epithelial phenotypes of GED: intestinal, gastric/foveolar, and hybrid type. The clinical significance of these differences needs to be investigated in future studies.

604 Mucin Phenotype and beta-Catenin Are Useful Markers To Predict Submucosal Invasion and Lymph Node Metastasis in Intestinal Type Early Gastric Cancer

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Background: With the increasing therapeutic use of endoscopic resection for intestinal early gastric cancer (EGC), it is very important to predict biological behaviors of intestinal type EGC prior to endoscopic resection. On this background, we tried to elucidate the presumptive clinicopathologic factors and biologic markers to predict submucosal invasion and lymph node metastasis based on mucin expression in EGCs.

Design: 130 cases of intestinal EGCs were divided as EGC-IPs (EGCs with intestinal mucin phenotypes) and EGC-GPs (ICs with intestinal mucin phenotypes) based on mucin expression. The expression of mucins (Muc2, Muc5Ac, Muc6, CD10), other protein markers (p53, CDX2, beta-catenin, E-cadherin, Smad4) by immunohistochemistry were studied.

Results: EGC-IPs showed significantly increased p53 expression, CDX2 expression and beta-catenin delocalization than EGC-GPs. Using binary logistic regression analysis, expression of both gastric mucin and nuclear beta catenin expression could be independent predictive factors of lymph node metastasis (p=0.004) and submucosal invasion (0.007) in intestinal EGCs. Lymphovascular emboli (p=0.001), and size of lesion (p=0.022) could be clinicopathological independent predictive factors of lymph node metastasis and submucosal invasion in intestinal EGCs respectively.

Conclusions: Taken together, we suggest that mucin phenotype and beta-catenin expression might be used to predict biologic behavior prior to endoscopic mucosal resection in intestinal EGC.

605 Diagnosis of Fibrosing Cholestatic Hepatitis C in Liver Transplant Recpients

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Background: Fibrosing cholestatic hepatitis C (FCH) is a rare atypical presentation of recurrent viral hepatitis C in transplant recipients, associated with immunosuppression. Clinically it is characterized by rapid recurrence of hepatitis and progressive liver failure. The current study is undertaken to assess the spectrum of histologic changes in our patient population and to validate a recently described scoring system for cholestasis and sinusoidal fibrosis.

Design: Clinical records of all Hepatitis C liver transplant recipients, at University of Wisconsin Hospital, between 1998-2007 were reviewed. Clinical cases of FCH (n = 11) were matched with controls (HCV+, non-FCH) by transplant year (n = 24). Two blinded pathologists evaluated a total of 38 biopsies from 35 patients and scored for portal inflammation, interface hepatitis, lobular necroinflammatory activity, bile duct damage, venous endothelialitis, parenchymal cholestasis, hepatocellular ballooning, bile ductular proliferation, inspissated bile plugs, portal/sinusoidal fibrosis. Cholestasis and sinusoidal fibrosis were scored using a recently described scoring system (Dixon LR et al, LT 2007).

Results: The median histologic activity index, median sinusoidal fibrosis and cholestasis scores were higher in FCH versus non FCH cases (p value <0.01 in each comparison). Serial biopsies in two FCH patients revealed progressively greater hepatocellular ballooning, cholestasis, ductular reaction and fibrosis. Three non FCH, HCV+ controls did have high cholestasis and fibrosis scores due to large duct obstruction, sepsis and drug toxicity respectively, important diagnostic considerations to be excluded before suggesting a diagnosis of FCH, in a transplant recipient presenting with early cholestasis, ballooning and sinusoidal fibrosis.

Conclusions: We found a spectrum of histologic changes in our post transplant FCH population similar to that previously described and were able to validate a recently described scoring system for cholestasis and sinusoidal fibrosis. The sinusoidal fibrosis score was felt to be a useful semi quantitative addition to assessment of fibrosis, reducing inter-observer subjectivity. Early recognition of FCH in post-transplant biopsies is vital to intervene early with antiviral chemotherapy, in an attempt to prevent graft loss.

606 Algorithm for Diagnosis of Gastrointestinal Stromal Tumor Using Immunohistochemstry of C-Kit and PDGFRA with Molecular Analysis

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Background: Gastrointestinal stromal tumor(GIST) is relatively rare disease, but the most common sarcoma in gastrointestinal tract. Immunoreactivity for c-kit helps to diagnose GIST with histologic features, positive vimentin and CD34 staining. Recently, many kinds of methods for the diagnosis of GIST have been developed including molecular diagnosis.

Design: Available 90 cases of GIST were selected in Department of Pathologoy of Kyungpook National University Hospital(KNUH) during 1998~2007. Tissue microarray(TMA) were made using core area of tumor tissues, which are representatives of the most cellular area. IHCs for c-kit and PDGFRA were done. And direct sequencing of hot spot exonal areas for c-kit and PDGFRA were performed from extracted DNAs of all 90 paraffin block tissue.

Results: Among 90 cases, 83.3%(75/90) were c-kit positive, 16.6%(15/90) were c-kit negative. And 93.3%(84/90) were PDGFRA positive, 6.6%(6/90) cases were PDGFRA negative. 15 cases of c-kit negative GISTs contained 1 case of PDGFRA negative, and 5 cases of PDGFRA negative GISTs showed c-kit positive. The 1 case, both c-kit and PDGFRA negative showed c-kit mutation in exon 11.

Conclusions: Combined with c-kit, immunohistochemical staining of PDGFRA is helpful for diagnosis of GIST. When both staining show negative immunoreactivity, c-kit mutation analysis for exon 11, 9 should be done first. And then PDGFRA mutation analysis for exon 12, 18 is recommended next. All GISTs are not need to examine mutational analysis first.

607 Molecular Features of Colonic Sessile Serrated Polyps with Dysplasia in Korea

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Background: Sessile serrated polyps with dysplasia (SSP-D), often referred to as "mixed" polyps, are believed to represent a precursor to serrated carcinomas. However, SSP-D usually contain either traditional serrated adenoma (SA) or conventional adenomatous (CA) type dysplasia, and it remains unclear which type represents a stronger precursor to cancer. The aim of this study was to evaluate molecular mutations in SSP-D, with particular attention to the different dysplasia subtypes.

Design: Routinely processed tissue from 55 SSP-D, including 22 with CA-type dysplasia, and 27 with SA-type dysplasia, and 6 hyperplastic polyps (HP) associated with SA-type dysplasia were evaluated for mutations of BRAF, KRAS, and for methylation of hMLH1, MGMT, and APC. Also evaluated were 16 serrated adenocarcinomas arising in SSP with dysplasia (11 with SA type dysplasia, 5 with CA type dysplasia) and 9 conventional adenocarcinomas as controls.

Results: 58.2%, 21.8%, 52.7%, 60%, and 36.4% of SSP-D contained BRAF or KRAS mutations, or methylation of hMLH1, MGMT and APC, respectively. In a comparison of dysplasia subgroups, BRAF mutations and hMLH1 methylation were significantly more common in SSP or HP with SA-type dysplasia (81.8% and 66.7%, respectively) compared to SSP-D with CA-type dysplasia (23% and 31%, p<0.000 and 0.008, respectively). No differences were observed within the polyp subgroups with regard to KRAS mutations or methylation of MGMT or APC. In SSP's with adenocarcinoma, BRAF mutation was significantly more common in those with a precursor SA component (18%) compared to a CA component (0%)(p=0.04), but no other differences were observed in any of the other genes. Control adenocarcinomas showed significantly increased APC mutations (78%) compared to the other groups (12.5%, p=0.004).

Conclusions: The molecular profile of SSP-D from Korea is different from those in Western countries. Frequent KRAS mutations found in SSP with CA-type dysplasia and serrated adenocarcinoma arising in SSP with CA type dysplasia support the hypothesis that KRAS-mutated SSP evolve from CAs, and that KRAS mutation is responsible for the serration in these polyps and carcinomatous transformation.

608 Molecular Features of Colorectal Hyperplastic Polyps from Korea

KM Kim, RD Odze. Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; Brigham and Womens Hospital, Harvard Medical School, Boston.

Background: Previous studies have shown specific lifestyle and dietary factors associated with an increased risk of development of hyperplastic polyps (HP). HP has also been shown to be related to the development of adenomas and colorectal carcinomas in some studies. Although studies of the molecular features of HP's have been performed in western countries, the molecular features of HP's in patients from the far east, such as Korea, which have a different lifestyle and diet, have never been performed.

Design: 112 HPs were retrieved from the surgical pathology files of Samsung Medical Center between 2004 and 2006 and classified as either microvesicular (MV) (n=89), goblet cell (GC) (n=13), mucin depleted (MD) (n=2), or indeterminate (Ind) (n=8) by 2 GI pathologists. In addition, 6 HP's associated with a serrated adenoma (SA) and 5 cases associated with intramucosal adenocarcinoma, were included. DNA from each case was evaluated for mutations of BRAF, and KRAS, and for methylation of hMLH1, MGMT, and APC.

Results: BRAF mutations were significantly more common in MV HP (63%) compared to the other subgroups (GC: 0%, others: 20%, p<0.001 for each comparison). In contrast, KRAS mutations were significantly more common in GC HP (77%) compared to all of the other polyp subgroups (p<0.001). The prevalence rate of hMLH1, MGMT, and APC methylation in HP's was 51.8%, 16.1%, and 38.4%, and there were no differences between the different HP subgroups. BRAF and KRAS mutations occurred in 83% and 17%, and hMLH1, MGMT, APC methylation occurred in 33%, 67% and 33% of SA's arising in HP. However, adenocarcinoma associated with HP showed no evidence of BRAF or KRAS mutations and hMLH1, MGMT, APC methylation occurred in 60%, 80% and 0% of cases.

Conclusions: In contrast to Western countries, both BRAF mutations and hMLH1 methylation are less frequent in HP from Korea. BRAF mutations are more common in GC HP, and MGMT methylation is more prevalent in MV HP. These results indicate that lifestyle and/or dietary factors may have a genetic and/or epigenetic influence on the development of HP's in different regions of the world.

609 Detection of H. Pylori by PCR in Gastric Biopsies

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Background: The detection of bacteria by conventional histology is considered the gold standard for the diagnosis of H. pylori infection in gastric biopsies. We recently showed, however, that in about 20% of biopsies with characteristic inflammatory pattern but no bacteria detected by conventional histology, PCR can reveal an infection by H. pylori (Zsikla et al.; Am J Surg Pathol 2006;30:242). To validate this data in the daily pathology practice, we prospectively analyzed all biopsies for the presence of H. pylori by PCR, which were considered by the signing out pathologist having an inflammatory pattern compatible with Helicobacter infection but lacking bacteria by histology.

Design: The histology of all biopsies which have been analyzed by nested PCR for Helicobacter bacteria at the Kantonales Institute of Pathology, Liestal, Switzerland between the 1. 1. 2003 to the 31.12.2007 were re-evaluated and the inflammation graded according to the Sidney system. A nested PCR for H. pylori was performed as previously described, including a control PCR to verify an adequate DNA extraction.

Results: Of the total of 360 biopsies tested, H. pylori was detected by PCR in 147 (40.8%) biopsies. The total inflammatory score was significantly higher in the PCR positive compared to the PCR negative biopsies (2.3 versus 2.8; p<0.05). In biopsies with an inflammatory score of ≥4, PCR for Helicobacter was positive in 48.1% compared to 38.8% of biopsies with a score of ≤ 3; however, this difference is not statistical significant. Only in biopsies with an inflammatory score of 1, a significantly lower detection rate was observed compared to the other inflammatory scores (18.6% versus 46.2%; p<0.0001).

Conclusions: Our data show that in gastric biopsies with an appropriate inflammatory pattern but lack of Helicobacter by histology, PCR detects H. pylori in about 40% of cases, confirming our previous data and validating this procedure in the setting of clinical practice. However, even in higher inflammatory scores, in more than 50% of biopsies the aetiology of the inflammation remains unclear.

610 Analysis of Incidence and Expression Levels of a Cancer-Specific CCK_2 Receptor Splice Variant in Gastrointestinal and Lung Tumors

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Background: The CCK_2 receptor (CCK_2R) is expressed in many gastrointestinal and lung tumors. Recently, it was described that in colorectal and pancreatic cancers a subset of CCK_2R corresponded to a splice variant with intron 4 retention (CCK_2R-i4). Unlike the wild-type receptor, the CCK_2R-i4 showed constitutive activity associated with increased tumor growth *in vitro*. Given the potential functional and clinical importance of this spliceoform, the aim of this study was to quantitatively characterize its occurrence in a broad collection of gastrointestinal and lung tumors.

Design: Eighty-one tumor samples, including insulinomas, ileal carcinoids, gastrointestinal stromal tumors (GIST), gastric, colorectal, and pancreatic ductal adenocarcinomas, cholangiocellular and hepatocellular carcinomas, small cell lung cancers (SCLC), non-SCLC, and bronchopulmonary carcinoids, as well as 21 samples of corresponding normal tissues were assessed for transcript expression of total CCK_2R, wild-type CCK_2R and CCK_2R-i4 with end-point and real-time RT-PCR, and for total CCK_2R protein expression on the basis of receptor binding with *in vitro* receptor autoradiography.

Results: Wild-type CCK_2R transcripts were present in the vast majority of investigated tumors and normal tissues, except for cholangiocellular carcinomas and normal lung. Conversely, the CCK_2R-i4 mRNA expression was restricted to specific tumor types, namely insulinomas (incidence 100%), GIST (100%), pancreatic carcinomas (14%), SCLC (67%), and non-SCLC (8%), and was not found in any normal tissues tested. CCK_2R-i4 transcript levels in individual tumors were very low, ranging from 0.02% of total CCK_2R transcripts in GIST to 0.14% in non-SCLC. Total CCK_2R transcript levels correlated fairly well with the amount of total CCK_2R protein as quantified with autoradiography (correlation coefficient r^2=0.658, p<0.001).

Conclusions: The CCK_2R-i4 is a marker of specific gastrointestinal and lung tumors. With its high selectivity for and high incidence in clinically important tumors like SCLC and GIST, the CCK_2R-i4 represents an attractive potential diagnostic or therapeutic target. The low CCK_2R-i4 transcript levels in these tumors predict that only highly sensitive diagnostic tests like PCR could prove clinically useful.

611 *CDKN2A* and *MTAP* Deletions in Peritoneal Mesotheliomas: Correlation with Loss of p16 Protein Expression and Survival

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Background: Homozygous deletion of *CDKN2A* is one of the most common genetic alterations in pleural mesotheliomas, occurring in 74% of cases. *MTAP* resides in the same gene cluster of the 9p21 region and is co-deleted in the majority of *CDKN2A* deleted cases. Correlation with p16 immunostaining is variable. This study examines the genetic alterations in peritoneal mesotheliomas, which may have a different pathogenesis than their pleural counterparts.

Design: 25 cases of peritoneal mesotheliomas in a triplicate tissue microarray were studied. Dual-color FISH was performed using a CEP9 probe and either a *CDKN2A* or *MTAP* locus-specific probe. Cases with >20% of nuclei lacking both signals for the locus-specific probe and having at least one signal for CEP9 were considered homozygous deleted. Cases with nuclear p16 immunostaining in <5% of cells were considered negative. Fisher exact tests were used unless specified.

Results: 8 of 25 (32%) peritoneal mesotheliomas had homozygous deletion of *CDKN2A*; *MTAP* was co-deleted and p16 protein expression was lost in every case.

Table 1.

	Number	M/F	Mean Age	Ep/Sarc	MTAP deletion	p16 loss
CDNK2A deletion	8	8/0	64	7/1	8	8
No CDNK2A deletion	17	13/4	51	16/1	1	5
		p = NS	p = 0.028 (T-test)	p = NS	p<0.00001	p = 0.001

Ep = epithelial; Sarc = sarcomatoid

Patients with *CDKN2A* deletions had worse overall and disease free survival (p=0.042 & p=0.018, respectively; Kaplan-Meier).

Conclusions: Similar to pleural mesotheliomas, patients with *CDKN2A* deletion had significantly worse survival. Detection of *CDKN2A/MTAP* co-deletion in peritoneal mesotheliomas coupled with a p16 stain as an inexpensive screening tool, can identify those patients with a worse prognosis and possible response to targeted therapy of the *MTAP*/AMP pathway.

612 Density of IgG4+ Plasma Cells in Ulcerative Colitis Correlates with the Degree of Inflammation and Not with Extraintestinal Manifestations

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Background: Ulcerative colitis (UC) is an immune-mediated inflammatory disease of the colon and often causes significant extraintestinal manifestations (EIM) such as primary sclerosing cholangitis and arthritis. A recently named disease entity, immunoglobulin subclass 4 (IgG4)-related systemic disease - originally described in patients with autoimmune pancreatitis, has been expanding to include inflammatory diseases in many organs with serum IgG4 as the marker of the disease activity. Approximately 10% of patients with IgG4-related autoimmune pancreatitis also have UC. While the nature of the relationship between UC and autoimmune pancreatitis is unknown, there has been speculation that a systemic deposition of IgG4 in both the pancreas and colon may be responsible. Thus, the objective of this study was to evaluate IgG4+ plasma cells in the colonic biopsies of patients with ulcerative colitis.

Design: Thirty eight patients with UC were identified and grouped into the following: (1) diagnostic biopsy with active disease (n=18), (2) surveillance biopsy with inactive disease (n=8), and (3) surveillance biopsy in patients with active disease and EIM (n=12). EIM in the samples included: primary sclerosing cholangitis, primary biliary cirrhosis, arthritis, ankylosing spondylitis and erythema nodosum. H&E and IgG4 (BioGenex, CA) immunostained slides were scored for disease activity and average number of IgG4+ plasma cells per 10 high power fields by two blinded reviewers.

Results: IgG4+ plasma cells in colonic biopsies of patients with ulcerative colitis were not significantly different when divided according to the presence and absence of EIM (p=0.62). However, IgG4+ plasma cells were significantly increased in biopsies showing severe active inflammation compared to biopsies showing mild active inflammation (p<0.05).

Group	Inactive*	Mild*	Moderate*	Severe*	Total*
1	0	1.6/6	4.4/5	3.7/7	3.2/18
2	1.5/8	0	0	0	1.5/8
3	0	1/4	2.4/7	10/1	2.6/12

*Average IgG4+ plasma cells per 10 hpf / number of biopsies

Conclusions: In colonic biopsies of patients with ulcerative colitis IgG4+ plasma cells are not useful in identifying the subset of UC patients with EIM. In this setting, the numbers of IgG4+ plasma cells are positively correlated with the degree of inflammation. Future directions include assessment of serum IgG4 level as a potential surrogate of disease activity in ulcerative colitis.

613 TFF-3 as an Early Biomarker of Neoplasia in Ulcerative Colitis

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Background: Biomarkers of neoplastic risk in ulcerative colitis (UC) are needed to focus surveillance efforts onto the patients most likely to benefit. Proteomics offers an excellent opportunity to identify new biomarkers that can be easily adapted to immunohistochemical screening tests.

Design: Quantitative proteomic profiling was performed by isotope-code affinity tag (ICAT) technology and tandem mass spectrometry using nondysplastic rectal tissue from UC with neoplasia elsewhere in the colon (progressor=P) in comparison to UC without dysplasia in the long term (nonprogressor=NP). Variably expressed peptides were validated by routine immunohistochemistry (IHC) on 2mm core tissue microarrays (TMA). Microarrays were constructed from 41 UC patients, including 27 NPs and 14 Ps (with the most advanced neoplastic diagnoses of low-grade dysplasia (LGD) in five patients, high-grade dysplasia (HGD) in four, and colorectal adenocarcinoma (CA) in

five). Nondysplastic rectal tissue from all patients was examined along with the full spectrum of available neoplastic lesions in each patient. IHC was graded for intensity and percentage of positive epithelial cells, each on a scale from 0-4, and with calculation of a final score as the product of the grades.

Results: Proteomics revealed TFF-3 down regulation in UC progressors. As shown in the table, TMA IHC confirmed a trend towards decreased TTF-3 expression in nondysplastic rectal biopsies from P versus NP patients. As also shown in the table, TTF-3 was progressively down regulated throughout neoplastic progression from nondysplastic epithelium to carcinoma ($p<0.05$) (see table).

	Nondysplastic Rectum - NP (n=27)	Nondysplastic Rectum - LGD (n=5)	Nondysplastic Rectum - HGD (n=4)	Nondysplastic Rectum - CA (n=5)	LGD (n=6)	HGD (n=9)	CA (n=5)
TFF-3 Score	13.4	11.4	16	9.1	6.9	6.5	3.2

Conclusions: Using ICAT proteomics and tandem mass spectrometry with IHC TMA validation, we demonstrate sequential downregulation of TFF-3 during progression from nondysplastic UC epithelium through dysplasia to CA. We further demonstrate a downward trend for TTF-3 in nondysplastic rectal mucosa from NP patients to CA patients. TFF-3 expression in colonocytes has been postulated to play a role in mucosal healing and to be protective against carcinogenesis. Our results offer further evidence of this and promise for TTF-3 IHC as a biomarker of neoplasia risk in UC.

614 Role of E-Cadherin and Beta-Catenin in Colorectal Cancer Progression

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Background: The adherens junction is the region of the cell membrane which is vital in the maintenance of normal cellular morphology. Reduced cellular adhesiveness facilitates cancer invasion and metastasis. E-cadherin forms an adherens complex in the cell membrane with beta-catenin and alpha-catenin. Beta-catenin may also translocate from the cell membrane to the nucleus where it acts in the dysregulation of the Wnt signalling pathway which plays an important role in colorectal cancer.

Design: The study cohort consisted of 109 sporadic colorectal cancers with full clinicopathological data. Microsatellite instability testing was performed using the National Cancer Institute consensus panel of five microsatellite markers. Tumors displaying instability in at least 2 of 5 markers were regarded as having high-level microsatellite instability (MSI-H). Expression of E-cadherin and beta-catenin was assessed by immunohistochemistry. E-cadherin was scored for membrane staining. Beta-catenin was scored for membrane, cytoplasmic and nuclear staining. E-cadherin gene promoter hypermethylation was investigated using methylation specific PCR. All results were correlated with patients' clinicopathological features.

Results: There was a strong positive association between E-cadherin gene promoter hypermethylation and decreased E-cadherin expression in the cell membrane ($p<0.001$). E-cadherin methylation also correlated with the presence of distant metastasis ($p<0.001$). Reduced expression of E-cadherin in the tumor cell membrane was associated with increased nuclear beta-catenin expression ($p=0.047$). Beta-catenin nuclear accumulation was indicative of distant metastasis ($p=0.046$). Membranous beta-catenin staining correlated with microsatellite unstable (MSI-H) tumors ($p=0.02$) and with right-sided location of tumors ($p=0.005$).

Conclusions: Loss of membranous E-cadherin and increased nuclear beta-catenin localization are associated with advanced colorectal cancer. The mechanism of loss of E-cadherin expresssion in advanced colorectal cancer appears to be silencing of the E-cadherin gene due to hypermethylation of its promoter.

615 Incidence of Lymph Node Metastasis from Early Gastric Cancer: Reappraisal of Japanese Criteria of EMR/ESD

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Background: With increasing use of endoscopic resection for therapeutic purpose in early gastric cancer (EGC) worldwide, it is very important to define strict criteria for EMR (endoscopic mucosal resection)/ESD (endoscopic submucosal dissection), due to most EMR/ESD criteria are based on Japanese data of lymph node (LN) metastasis in EGC.

Design: We tried to review incidence of LN metastasis in 485 EGC patients underwent gastrectomy with LN dissection at the Pusan National University Hospital during four years (275=mucosal(m) EGC, 210=submucosal(sm) EGC). Various clinicopathologic factors were analyzed with status of lymph node metastasis.

Results: Overall, there are 14.02% (68/485) of LN metastasis in EGC (mEGC=5.3% (14/275), smEGC=25.7% (54/210). Increased size, gross type (elevated), depth of invasion and lymphovascular tumor emboli are associated with LN metastasis in EGC. In 275 cases of mEGC, only lymphovascular emboli is associated with LN metastasis. In smEGC, size, depth of invasion and lymphovascular emboli are related to LN metastasis. Even there was LN metastasis (12/496, 4.05%) within EMR/ESD criteria by Japanese data (intestinal, sm1 invasion, no lymphovascular tumor emboli, less than 3cm in size).

Conclusions: Taken together, we recommend that more worldwide survey of LN metastasis of EGC is needed to define strict criteria of EMR/ESD for therapuetic purposes.

616 Aberrant Methylation of DNA-Mismatch Repair Genes in Elderly Patients with Sporadic Gastric Carcinoma: A Comparison with Young Patients

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Background: Epigenetic silencing of DNA repair genes by promoter DNA methylation is observed in various carcinomas including gastric carcinoma. Although most gastric carcinomas occur in elderly patients, almost 10% of patients present before 45 years of age. These younger patients are believed to have different genetic and epigenetic profiles from those patients with sporadic carcinomas occurring at a later age. The study was aimed to explore differences in epigenetic alteration of DNA mismatch repair genes in relation to age of onset of gastric carcinoma.

Design: Two study groups consisted of 100 elderly patients (age >75 years; mean age 84.1 years) and 100 young patients (age < 45 years; mean age 38.1 years). Aberrant DNA methylation of four mismatch repair genes, *hMLH1, hMSH2, hMSH3,* and *MGMT* were compared by bisulfite modification and methylation specific PCR (MSP). The expression of *hMLH1, hMSH2, hMSH3,* and *MGMT* gene product was also examined by immunohistochemistry.

Results: Among 100 young patients with gastric carcinoma, CpG island methylation was found in 2.0% for *hMLH1*, 14.0% for *hMSH2*, 7.0% for *hMSH3*, and 24.0% for *MGMT*. Among the 100 elderly patients, methylation was found in 27.0% for *hMLH1*, 13.0% for *hMSH2*, 18.0% for *hMSH3*, and 26.0% for *MGMT*. Methylation frequencies for *hMLH1* and *hMSH3* were significantly higher in elderly than young patients with gastric carcinoma ($p<0.001$ and $p=0,013$, respectively). Immunohistochemically, loss or reduced nuclear expression of hMLH1, hMSH2, hMSH3, and MGMT protein was seen in 40 (20.0%), 39 (19.5%), 94 (47.0%), and 80 (40.0%) of 200 patients with gastric carcinoma, with a significant correlation between aberrant *hMLH1 and MGMT* methylation and loss of *hMLH1* and MGMT protein expression ($p<0.001$ and $p<0.01$, respectively). The prevalence of aberrant *hMLH1* and *hMSH3* methylation increased significantly with age among patients (Cochran-Armitage test for trend, $p=0.016$). However, the correlation between methylation status and clinicopathologic characteristics was insignificant based upon univariate and multivariate analysis.

Conclusions: These results suggest that methylation of *hMLH1* and *hMSH3* is age-related and may play an important role in gastric carcinogenesis in the elderly.

617 Inherited Lack of Death Receptor 4 (DR4) Expression in Gastric Carcinoma through Gene Promoter Methylation

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Background: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anti-cancer agent because of its selective toxicity in cancer cells. TRAIL sensitivity is suggested to be affected by the expression balance between pro-apoptotic death receptors (DR4 and DR5) and anti-apoptotic decoy receptors (DcR1 and DcR2). Promoter methylation status of death receptors in gastric cancer has never been studied in a large series. In this study we investigated the frequency of promoter methylation in *DR4*, *DR5*, *DcR1*, *DcR2* and *caspase-8*, the next signaling step, in early and advanced gastric carcinoma.

Design: Promoter methylation of *DR4*, *DR5*, *DcR1*, *DcR2* and *caspase-8* was evaluated in 62 early gastric cancers (EGCs) and 60 advanced gastric cancers (AGCs) from 122 patients who had undergone gastrectomy. Methylation status was also examined in normal mucosa samples from 24 patients with benign gastric pathology. The methylation status of five gene promoters was determined by bisulfite modification and methylation-specific PCR (MSP).

Results: Sixty one (98.4%) cases of 62 EGCs and 50 (83.3%) cases of AGCs exhibited promoter methylation of *DR4*. Of the 62 EGCs, 1 (1.6%), 7 (11.3%), 10 (16.1 %) and 12 (18.3%) cases were methylated for *DR5, DcR1, DcR2* and *Caspase-8*. Promoter methylation was seen in 0 (0%), 6 (10.0%), 14 (16.7%) and 10 (16.0%) cases of 60 AGCs for each promoter. Interestingly, 24 (100%) cases of 24 normal samples showed promoter methylation of *DR4*. With other genes, 0 (0%), 4 (16.05), 5 (20.8%), 1 (4.2%) cases of 24 normal samples demonstrated methylation for each gene promoters. Hypomethylation rather than hypermethylation of *DR4* was observed in the progression of gastric tumor to more invasive carcinoma. Difference between the frequencies of other gene promoter methylation was not statistically significant ($p>0.05$, respectively).

Conclusions: We authors showed high promoter methylation rate of *DR4* in gastric carcinoma in relation with its constituitive methylation in gastric normal mucosa. Lack of gene expression through promoter methylation in proapoptotic death receptors may contribute to TRAIL insensitivity of gastric cancer. Knowledge on the promoter methylation status of death receptors in gastric carcinoma in the present study will contribute to new, patient-tailored, treatment strategies for gastric cancer patients.

618 EUS-FNA and Cyst Fluid Analysis in the Evaluation of Pancreatic Cystic Lesions: A Retrospective Study of 197 Cases

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Background: Pancreatic cystic lesions are being increasingly identified. The common cystic lesions of the pancreas include pseudocysts (PC) and cystic neoplasms, which include serous cystadenoma (SCA), mucinous cystadenoma (MCA), intraductal papillary mucinous neoplasm (IPMN), and adenocarcinoma with cystic degeneration (ACA). Benign cystic lesions (PC and SCA) are generally managed nonoperatively, while both premalignant (MCN and IPMN) and malignant (ACA) neoplasms require surgical resection. About 80-90% of the pancreatic cysts are PC with the remaining 10% to 20% being cystic neoplasm. Accurate diagnosis of pancreatic cystic lesions is critical for selecting treatment options. Previous studies involving endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) and cyst fluid analysis have been encouraging, but

their role in the evaluation of pancreatic cystic lesions needs to be further elucidated.
Design: The records of 197 patients underwent EUS in our hospital from 2000 to 2006 were reviewed. Available data of cyst fluid analysis (CEA, CA 19-9, amylase, and lipase) were reviewed. FNA result was compared to final surgical pathology diagnosis if surgery was performed.
Results: Among the 197 patients (101 women, 96 men; age: 11-88 years), 81 had cystic lesion in the pancreatic head, 51 in the pancreatic body, 39 in the pancreatic tail, and 7 had multiple cysts involving at least 2 sites. The cyst size varied from 0.8 cm to 17 cm. Thirty-three patients who had FNA further underwent surgical resection and presented the following histopathologic correlates: 4 PC, 6 SCA, 5 MCA, 7 IPMN, 1 solid pseudopapillary neoplasm, 5 ACA, 1 fibromatosis, 1 marked epithelial atypia, 1 metastatic melanoma, and 2 neuroendocrine tumors. EUS-FNA accurately diagnosed 26 of 29 cases (90%) with insufficient specimen in 4 cases. The sensitivity and specificity of EUS-FNA in our group were 87.5% and 100% respectively. Surprisingly, spindle cells were present in the cytology smears in 4 of 4 cases of MCA. Cyst fluid analysis showed that CEA level was significantly higher in ACA (30,009 ng/mL) vs PC (134 ng/mL)(p=0.043), while amylase level was higher in PC (22710 U/L) compared with ACA (7519 U/L).
Conclusions: Our study showed that EUS-FNA plus cyst fluid analysis is a very sensitive and specific modality for accurate diagnosis of pancreatic cystic lesions. In addition, the presence of spindle cells in cytology smear is an important diagnostic feature for MCA which has not been identified previously.

619 Mitochondrial DNA 4977 Basepair Deletion Occurs More Frequently in Normal Colonic Mucosa Than in Tubular Adenoma: Its Potential Role as Tumor Suppressor in Progression of Colon Cancer

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Background: Tubular adenoma of the colon has long been established as a precursor of colonic adenocarcinoma. The molecular progression of colorectal carcinoma has been established and shows some common genetic alterations shared by both tubular adenomas and colorectal carcinoma. In this study, we aim to characterize the presence and frequency of a common human mitochondrial DNA deletion, delta mtDNA 4977, in tubular adenoma and normal colonic mucosa. Mitochondria are the primary source of free radicals due to their active roles in oxidative phosphorylation, which in turn causes mitochondrial DNA damage (mutation or deletion) and dysfunction. Mitochondrial DNA deletions occur frequently in the process of aging and malignant transformation. The most frequently occurred and commonly analyzed human mitochondrial DNA deletion is delta mtDNA 4977. This is a 4977 basepair mtDNA deletion between two 13-basepair direct repeats.
Design: A total of 12 cases of normal colonic mucosa and 10 cases of tubular adenoma were included in the study. Tissue sections were used for microdissection and DNA samples from these tissues were extracted and subjected to PCR amplification using a primer set that flanks the breakpoint of delta mtDNA 4977. The primer sequences are as follow: MtDNA 8342 forward: 5'-gaa cca aca cct ctt tac ag-3' and MtDNA 13524 reverse: 5'-gat gat gtg gtc ttt gga g-3'.
Results: Delta mtDNA 4977 was detected in 8 of 12 (66.6%) cases of normal colonic mucosa and 1 of 10 (10%) cases of tubular adenoma of the colon.
Conclusions: Delta mtDNA 4977 occurs much more frequently in normal colonic mucosa (66.6%) than in tubular adenoma (10%). It has been hypothesized that delta mtDNA4977 is intolerable and thus, is less commonly seen in transformed cells, probably due to higher metabolism rate and increased free radical generation. When cells that initially had delta mtDNA4977 progress to more proliferative adenomatous epithelia in tubular adenoma, the deletion may confer a metabolic disadvantage to these cells. Cells with such a mtDNA deletion may be overgrown by other neoplastic cells without this mtDNA deletion. Our results support a tumor-suppressive role of Delta mtDNA 4977 in colorectal carcinogenesis.

620 Esophageal Adenocarcinomas Overexpress MicroRNA-21: A Tissue Microarray Study of 64 Cases Using In-Situ Hybridization

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Background: MicroRNAs (miR) are short naturally occurring single-stranded RNA molecules that regulate expression of target genes post-transcriptionally. They have been implicated as key regulatory molecules in tumors where they can function as both tumor suppressors and oncogenes. MiR-21 is one of the most commonly deregulated miR in many malignancies. We compared expression of miR-21 in esophageal adenocarcinoma (Ad) and Barrett's esophagus without (B) and with dysplasia (BD) to investigate miR-21 in progression of Barrett's esophagus to adenocarcinoma.
Design: Archival files from our institution were searched for esophageal Ad resection specimens, and cases were reviewed for Ad, B, BD, and normal glands (NG). These areas were cored from formalin-fixed, paraffin embedded blocks (2 cores per block) and were arrayed to create a tissue microarray of cores 2.0 mm each. Sections were pre-hybridized with Exiqon hybridization buffer. Exiqon DIG-labeled miRCURY LNA probes of miR-21, U6, and Scramble were then added to the slides at a 50nM and hybridized for 20 hours and detected using alkaline phosphatase conjugated anti-DIG Fab fragment (probes provided by Exiqon). Controls stained appropriately and slides were interpreted by 2 pathologists. MiR-21 was graded as negative, weakly positive (1+) or strongly positive (2+).
Results: 64 Ad (5 well, 35 moderate, 24 poorly differentiated) were identified with sufficient tissue for evaluation of B in 20 and BD in 14.

MiR-21 Expression

	Number	Negative	1+	2+
Normal Glands	43	40 (93%)	3 (7%)	0
Barrett	20	16 (80%)	4 (20%)	0
Barrett Dysplasia	14	2 (14%)	12 (86%)	0
Adenocarcinoma	64	7 (11%)	36 (56%)	21 (33%)

MiR-21 was overexpressed in Ad (89%, 57/64) and BD (86%, 12/14) with strong staining in Ad (33%, 21/64) and not in BD. Weak expression of miR-21 was seen in NG and B in 7% (3/43) and 20% (4/20), respectively. MiR-21 expression was significantly increased in Ad and BD as compared to B and NG ($p<0.005$). There was no significant difference in miR-21 expression among the grades of Ad.
Conclusions: MiR-21 is overexpressed in Ad and BD as compared to B or NG suggesting that miR-21 has a role in the progression of Barrett's esophagus to Ad. Although the percent of areas with overexpression of miR-21 was similar in Ad and BD, strong staining was only seen in Ad further supporting the role of miR-21 in carcinogenesis. Additional studies are necessary to evaluate the association of miR-21 with outcome.

621 Experience in KRAS Mutational Analysis for Colorectal Cancer

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Background: Colorectal cancer (CRC) is the third leading cause of cancer-related death in the Unites States. *KRAS*, an oncogene, has been reported to be mutated in about 30% of CRCs. Emerging data strongly suggest that mutations in *KRAS* in CRCs are predictive of resistance to epidermal growth factor receptor (EGFR) inhibitor treatment. *KRAS* mutation analysis will be important before patients start EGFR inhibitor treatment. *KRAS* mutation has been suggested to be associated with poor prognosis, well to moderate differentiation, diffuse proliferation, and lack of apoptosis in CRCs. However, no detailed morphology comparison between *KRAS* mutation positive and wild type CRCs has been reported. This study sought to determine 1) the frequency of *KRAS* mutation and the most common *KRAS* mutation types in advanced CRCs and 2) the morphologic features associated with *KRAS* mutation positive CRCs.
Design: 89 cases of CRCs were included and formalin-fixed parraffin-embedded tissue sections were used for microdissection and DNA extraction. PCR was performed for a 263 basepair fragment, and the PCR product was subjected to cycle sequencing. The frequency and the types of *KRAS* mutations were determined. A subset of tumors were used for a detailed blinded histologic review. Features assessed included dirty necrosis, tumor differentiation, mitotic activity, apoptotic activity, mucinous component, tumor infiltrating lymphocytes, and complexity of malignant glands. The morphologic features were correlated to the *KRAS* mutational status. Fisher Exact test was used to analyze the data.
Results: The specimens tested included 86 primary carcinomas, and 3 metastatic CRCs. 22 specimens were found to harbor a *KRAS* mutation (24.7%). The mutations were distributed between codon 12 (12 cases; 54.5%) and codon 13 (10 cases; 45.57%). The types of mutation include GGT>TGT (3), GGT>GAT (6), GGT>GTT (3) in codon 12 and GGC>GAC in codon 13 (10). Histologic slides from 12 cases of *KRAS* mutated CRCs and 25 cases of wild type CRCs were reviewed. The histologic features that correlated with *KRAS* mutation were mitotic activity and apoptotic bodies (p=.045 and p=.028, respectively).
Conclusions: *KRAS* mutation occurs approximately in 24.7% of CRCs. The most common mutation types are GGT>GAT in codon 12 and GGC>GAC in codon 13, which accounted for 72.7% of the mutants. Tumors with mutated *KRAS* appears to be more mitotically active and undergo robust apoptosis.

622 SOX2 Is Highly Expressed in Squamous Cell Carcinomas of the Gastrointestinal Tract

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Background: SOX2 is an HMG-box embryonic stem cell transcription factor that is expressed in the developing foregut and normal adult gastric epithelium and is down-regulated in intestinal metaplasia of the stomach and esophagus. In addition, SOX2 co-localizes with p63 in the basal layer of the esophageal squamous epithelium, and plays a critical role in the maintenance of this stratified squamous epithelium. Recent studies have demonstrated that SOX2 is rarely detected in intestinal-type gastric and colonic adenocarcinomas (ACA), where it shows an inverse correlation with CDX2. SOX2 expression in squamous cell carcinomas (SCC) of the gastrointestinal tract has not previously been evaluated. Therefore, the purpose of this study was to determine whether SOX2 is differentially expressed in SCC versus ACA of the esophagus and anorectum and to compare its expression to p63 and CDX2.
Design: In total, 70 tumors were evaluated: 26 esophageal SCC, 23 esophageal ACA, 10 SCC of the anal canal, and 11 rectal ACA. Immunohistochemistry for SOX2 (Chemicon; polyclonal; 1:1500 dilution), p63, and CDX2 was performed. Staining in >5% of nuclei was considered positive. Statistical analysis was performed using the Fisher exact test; all reported p values are two-tailed.
Results:

Immunohistochemistry Results

Tumor type (N)	SOX2	p value	p63	p value	CDX2	p value
	# (%)		# (%)		# (%)	
Esophageal SCC (26)	21 (81%)	<0.0001	25 (96%)	<0.0001	2 (8%)	<0.0001
Esophageal ACA (23)	3 (13%)		0 (0%)		17 (74%)	
Anal canal SCC (10)	9 (90%)	0.002	10 (100%)	<0.0001	0 (0%)	<0.0001
Rectal ACA (11)	2 (18%)		0 (0%)		10 (91%)	

Of note, only one poorly differentiated ACA expressed SOX2; this tumor was diffusely positive for CDX2. The single esophageal SCC that was negative for p63 was strongly positive for SOX2.

Conclusions: SOX2 is preferentially expressed in SCC of the esophagus and anal canal. Few ACA from these anatomic sites are positive for SOX2. SOX2 may be useful in an immunohistochemical panel along with p63 and CDX2 to differentiate between SCC and ACA of the gastrointestinal tract.

623 Selected MicroRNA Species Are Overexpressed in Late Stage and Microsatellite Unstable Colorectal Cancers

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Background: MicroRNAs (miRNAs) are small noncoding RNAs involved in the post-transcriptional regulation of gene expression, and their aberrant expression has been implicated in colorectal carcinogenesis. In this study, we assessed a series of colorectal adenocarcinomas (CRC) for potential relationships between expression of selected miRNAs, microsatellite status, and pathologic stage, in order to determine whether these markers varied among biologically different tumors.

Design: Eighty-one CRCs were pathologically staged and 72 of them were assessed for microsatellite instability (MSI) using Bethesda panel. All cases were evaluated for expression of 7 miRNAs via quantitative RT-PCR, including 5 (*miR-20a*, *miR-21*, *miR-106a*, *miR-181b* and *miR-183*) and 2 (*miR-145* and *miR-192*) that were previously shown to be increased and decreased in CRC, respectively. Control amplifications of small RNA U6 were performed for data normalization. The miRNA levels were compared between stage I, II, III, and IV tumors using ANOVA and Tukey's test. Microsatellite stable (MSS) and unstable (MSI-H) tumors were compared using the non-parametric Wilcoxon rank sum test. Correlation coefficients between miRNA pairs were also calculated.

Results: There were 11 stage I, 23 stage II, 31 stage III and 16 stage IV tumors in this series, which included 56 MSS and 16 MSI-H cancers. Four miRNAs (*miR-20a*, *miR-183*, *miR-106a*, and *miR-21*) showed a trend toward increased expression in stage IV tumors (p=0.01 to 0.07). These same 4 miRNAs, as well as *miR-181b* and *miR-192*, also showed significantly higher expression in MSI-H than in MSS tumors ($p<0.05$ for all comparisons). Interestingly, *miR-20a* was most significantly overexpressed both in stage IV tumors and in MSI-H tumors, compared to lower-stage and MSS tumors (p=0.01 and p=0.007, respectively). With the exception of miR-145, moderate to strong correlations (r=0.46 to 0.80) were observed between all miRNA pairs, especially between *miR-20a*, *miR-21* and *miR183* (r=0.76 to 0.80).

Conclusions: Concerted over-expression of *miR-20a*, *miR-183*, *miR-106a*, and *miR-21* occurs in colorectal carcinomas of advanced stage. These markers, in addition to *miR-181b* and *miR-192*, are also increased in MSI-H compared to MSS carcinomas, irrespective of pathologic stage. These findings indicate that miRNAs are differentially expressed in CRCs that develop via different pathways and analysis of their expression may provide prognostically important information.

624 High Frequency of Loss of Heterozygosity in Colorectal Signet Ring Carcinomas Compared to Mucinous and Nonmucinous Adenocarcinomas

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Background: Signet ring cell carcinoma is a rare subtype of colorectal cancer associated with a poor prognosis.This study compares the molecular features of SRC with mucinous (MC) and nonmucinous adenocarcinoma(AC).The relationship of molecular abnormalities with survival in SRC is also examined.

Design: Microsatellite instability (MSI) and methylation status was determined in SRC (n=33),MC(n=26) and AC(n=57).For MSI, the 5 Bethesda markers were used; instability at >30% of markers was considered MSI.For methylation analysis,methylation specific PCR was used at 7 loci: *hMLH1,p16,RASSF2, HIC1,MINT1,MINT31,MGMT*. Methylation at 3 or more loci was considered CIMP+ and the remaining as CIMP-negative.Loss of heterozygosity(LOH)analysis was done at 4 loci: 5q,8p,17p,18q. Tumors with LOH at any locus were considered LOH+.BRAF and KRAS mutations were determined by PCR followed by sequencing.

Results: MSI,CIMP+ status and BRAF mutations were more often seen in SRC and MC compared to AC (see table).LOH status was available in 15 SRC and was present in 14 (94%) cases,including 5(100%)MSI cases and 9(90%)microsatellite stable(MSS)tumors. In SRC, BRAF and KRAS mutations were present in 33% and 52% respectively.BRAF mutations were significantly associated with CIMP+ status(p=0.002).BRAF mutations did not have any impact on overall survival.However, the 5-year survival was zero in patients with MSS cancers and BRAF mutations compared to 100% in MSI cancers with BRAF mutations(p=0.01).

	MSI	LOH	MSI withLOH	CIMP+	BRAF mutation	KRAS mutation	5-year survival
SRC (n=33)	24	93	100	48	33	52	31
MC (n=26)	27	60	14	38	46	27	50
AC (n=57)	12	71	14	18	16	40	58
p value	0.2, 0.08	0.02, 0.04	0.008, 0.008	0.3, 0.002	0.3, 0.04	0.05, 0.2	0.07, 0.02

All figures are precentages. p values reflect SRC vs MC and SRC vs AC

Conclusions: Loss of heterozygosity is more frequent in SRC compared to MC and AC. In contrast to MC and AC, LOH is observed in all MSI+ SRC.This may explain its aggressive behavior irrespective of MSI status.BRAF mutations and CIMP+ status are similar in SRC and MC but more frequent compared to AC.In SRC, BRAF mutations adversely affect survival in MSS,but not in MSI tumors.The high frequency of methylation and BRAF mutations suggests that many SRCs may be related to the serrated pathway of carcinogenesis.

625 Loss of Neutrophil Gelatinase Associated Lipocalin (NGAL) Expression in Esophageal Adenocarcinoma (EAC) Is Associated with Higher Pathologic Stage and Poor Survival

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Background: NGAL is a glycoprotein which modifies genes important in cellular differentiation and shown to be abnormally expressed in colon and pancreatic carcinomas and has not been studied in EAC.

Design: Tissue microarray (TMA) from resection specimens of 118 patients (1986-1998) with clinically localized esophageal adenocarcinoma who underwent esophagectomy without preoperative adjuvant therapy were studied. Normal squamous and gastric mucosa (n=5) were used as control. Immunostain for NGAL was performed (monoclonal Ab, Assay Designs, USA) on the TMA following antigen retrieval and blocking of endogenous peroxidase activity. The staining analysis was performed by the Ariol Automated Digital Imaging System (Genetix Limited,UK). A computer software was used to derive quantitative measures of tissue area of chromogen staining and staining intensity. We considered the number of positive tumor cells with any staining intensity. We graded the immunohistochemical stains as grade 1, <10%; grade 2, 10-25%; grade 3, 25-50%; and grade 4, >50%; of the cells in the total area. The staining grades were compared with different pathologic parameters and survival outcome.

Results: The study included 107 men and 11 women with average age of 63 years. All controls specimens showed grade 4 staining intensity. Table shows the correlation of NGAL expression with pathologic parameters and survival.

Table

Characateristics	NGAL Grade 1	NGAL Grade 2	NGAL Grade 3	NGAL Grade 4	p value
Tumor Differentiation					0.106
Well (n=5)	1	0	1	3	
Moderate (n=49)	15	9	6	19	
Poor (60)	30	9	9	12	
Location					0.02
Upper/Mid esophagus (n=6)	1	3	2	0	
Lower Esophagus (n=112)	46	16	15	35	
pT stage					0.001
T1/T2 (n=14)	1	1	1	11	
T3/T4 (n=104)	46	18	16	24	
pN stage					0.000
N0 (n=31)	1	0	3	27	
N1(n=87)	46	19	14	8	
pM stage					0.002
M0 (n=100)	34	16	15	35	
M1 (n=18)	13	3	2	0	
Overall Survival(months)					0.000
Mean±SD (n=118)	20.43±3.7	18.34±3.8	22.15 ±5.5	84.11 ±12.25	
Median (n=118)	12.87	11.03	15.03	60.33	
Recurrence					0.018
No (n=55)	19	6	6	24	
Yes (n=63)	28	13	11	11	

Conclusions: This study of large cohort of uniformly treated patients with EAC demonstrates loss of NGAL expression is a marker of aggressive behavior in EAC.

626 microRNA-196A: A Potential Marker of Progression in Barrett's Esophagus-Dysplasia-Adenocarcinoma Sequence

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Background: The low yield and high cost of endoscopic screening for Barrett's esophagus (BE) necessitates a need for novel biomarkers like microRNA (miR) in progression of BE. We recently showed high miR-196a in esophageal adenocarcinoma (EA). In this study we evaluated miR-196a as a marker of progression in BE-dysplasia-EA sequence and correlated miR-196a with it's *in silico* predicted targets in EA.

Design: miR-196a levels were measured in microdissected samples of normal squamous (NSM), BE (intestinal metaplasia), low-grade dysplasia (LGD) and high-grade dysplasia (HGD) from formalin fixed tissue of surgically resected specimens of 10 stage I EA patients, by stem loop real-time qPCR using TaqMan minor groove binding probe (Applied Biosystems, CA). miR-196a levels were measured from EA in 5 of 10 patients. miR-16 was used as a normalizer and miR-196a expression was measured by comparative CT method ($2^{-\Delta CT}$). Next, we correlated miR-196a with mRNA levels of its targets; keratin 5 (KRT5), small proline-rich protein 2C (SPRR2C), S100 calcium binding protein A9 (S100A9), that are downregulated during progression of BE, in frozen samples of additional 10 patients of EA. For cDNA synthesis 200 ng of RNA was reverse transcribed and real time qPCR was performed with TaqMan minor groove binding probe and ABI Prism7900 HT sequence detection system (PE Applied Biosystems). Statistical analysis was performed using SPSS(SPSS, IL).

Results: The population for progression analysis had 10 men (age:65 yrs). The mean (M)±SD miR-196a levels were 0.0005±0.0007 in NSM, 0.0140±0.015 in BE, 0.013±0.009 in LGD, 0.030±0.016 in HGD and 0.0790±0.058 in EA. The differences among each stage were statistically significant by one-way within subjects ANOVA with $p < 0.0001$. The pair wise comparison for miR-196a levels in each lesion was significantly higher than control NSM: NSM vs. BE (p=0.00001) vs. LGD (p=0.001), vs. HGD (p=0.0006) vs. EA (p=0.00005). The population for target correlative study included 9 men and 1 woman (age:62 yrs) and stage II or III disease. The M±SD miR-196a level in EA (0.025±0.009) was higher than NSM (0.00047±0.00067). The M±SD mRNA levels of SPRR2C, S100A9 and KRT 5 were 23.46±38.36, 246.4±359.3, 29±36.87 and correlated inversely with miR-196a levels ($p<0.01$).

Conclusions: miR-196a is a potential marker of progression of BE-dysplasia-EA sequence and KRT5, SPRR2C and S100A9 are targets of miR-196a in EA.

627 Cytochrome *C* Oxidase-I Deficient Foci in Ulcerative Colitis: A Novel Surrogate Biomarker of Early Carcinogenesis

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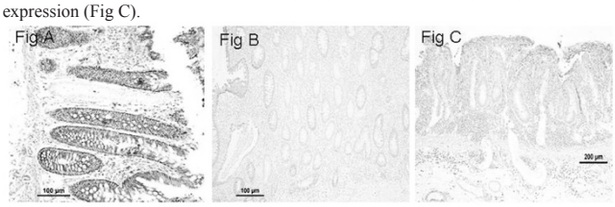
Background: Oxidative stress has been implicated in the pathogenesis of inflammatory bowel disease (IBD) and its progression to colorectal carcinoma. Cytochrome oxidase *c* subunit I (CcOI) is a key mitochondrial enzyme involved in apoptosis regulation. Experimental data show extensive positive expression of CcOI in normal tissues with loss of expression associated with its gene mutation and mutant cells displaying a clonal growth pattern. Thus, CcOI may be a unique biomarker for assessing oxidative stress-induced gene mutations, clonal cell growth, and carcinogenesis. Our study explored the potential of CcOI as an early biomarker of IBD-associated carcinogenesis by comparing CcOI expression patterns in ulcerative colitis (UC) to normal colon.

Design: 70 colectomy specimens from patients with varying degrees of involvement by UC, and 5 segmental resections for diverticulosis of normal colon were examined for CcOI expression patterns. Immunohistochemistry was performed for CcOI on each case. Normal colon served as a positive control.

Results: In normal colon, extensive positive staining for CcOI was seen in epithelial and stromal cells. Rare single glands showed loss of CcOI expression (Fig A). CcOI-deficient foci were defined as consecutive glands showing ≥50% staining loss mainly in the crypts and were categorized as either macrofoci (≥5 glands) (Fig B) or microfoci (<5 glands) (Fig A). CcOI-deficient foci were quantitated as number of foci per 100 glands. In normal colon, no macrofoci and only occasional microfoci were identified; greater than 95% of foci were single glands only. In UC specimens both macro and microfoci of CcOI loss were identified. The mean number of macrofoci/100 glands was 0.45+/-0.5, and the mean number of microfoci/100 glands was 0.3+/-0.8, significantly less than in control colon. 8 cases of UC (11%) showed extensive (>50%) loss of CcOI expression (Fig C).



Conclusions: Our results indicate that CcOI-deficient foci may serve as a useful surrogate biomarker of early carcinogenesis in patients with inflammatory bowel disease, particularly for early disease detection and prevention in this patient population. It also warrants investigating the biologic events in these foci in future.

628 Isolated Eosinophilic Gastritis: A Distinct and Clinically Unrecognized Variant Causing Giant Rugal Folds

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Background: Eosinophilic gastroenteritis (EG) encompasses a spectrum of diseases characterized by eosinophilic infiltration of one or more segments of the gastrointestinal tract. Patients may or may not have atopy or peripheral blood eosinophilia. When patients present with upper abdominal symptoms and undergo esophagogastroduodenoscopy (EGD), the presence of giant rugal folds is endoscopically and clinically suspicious for several disorders, including proton pump inhibitor-related hyperplasia, linitis plastica type diffuse carcinoma, Menetrier's disease, Zollinger-Ellison syndrome and lymphoma. However, occasionally such a presentation is due to clinically unsuspected isolated eosinophilic gastritis. We report five such cases of this variant.

Design: We searched all upper endoscopic biopsies (EGD) from January 2006 to August 2008 (32 months), in our pathology information system for giant rugal folds of stomach and diagnoses of "eosinophilic gastritis" and "eosinophilic gastroenteritis". We identified five patients with isolated eosinophilic gastritis (2 M, 3 F; 3-61 years, mean age 34). We reviewed the clinical history, presenting symptoms, endoscopic findings, peripheral blood eosinophil counts and histopathology.

Results: All five patients (2 M, 3 F; 3-61 years, mean 34) presented with abdominal pain, nausea and vomiting. Only one patient had known atopy and peripheral blood eosinophilia. Interestingly, all five patients had endoscopic giant gastric rugal folds and dense lamina propria eosinophilia isolated to the stomach. Five other patients (4 M, 1 F; 5-60 years, mean age 33) had clinically recognized eosinophilic gastroenteritis with atopy (3/5) and mild peripheral blood eosinophilia (4/5). They presented with abdominal pain and/or weight loss, had a mild to moderate diffuse increase in eosinophils in the stomach and duodenum and endoscopic gastritis without giant rugal folds.

Conclusions: Isolated eosinophilic gastritis must be considered as a potential cause for giant rugal folds apart from the more common diseases associated with giant gastric rugal folds. This appears to be a variant of eosinophilic gastroenteritis with distinct characteristics such as dense eosinophilic infiltrate (likely contributing to the large folds), isolated involvement of the stomach and clinical non-recognition with less obvious atopy and peripheral blood eosinophilia.

629 Endoscopic Ultrasound (EUS)-Guided Fine Needle Aspiration (FNA) Cytology of Gastrointestinal Stromal Tumors: A 15-Year Retrospective Study of 104 Cases

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Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Due to their submucosal location within the GI tract, they are difficult to diagnose by conventional surgical biopsy techniques. EUS-guided FNA has been increasingly used for the preoperative diagnosis of GISTs. In reviewing our experience, our aim in this study is to determine the accuracy of EUS-guided FNA for the diagnosis of GIST.

Design: A computerized search of the cytopathology laboratory information system was performed and all cases in which a diagnosis of GIST was rendered or suggested by FNA were identified. All correlating surgical pathology reports were obtained and all cytology slides and correlating surgical pathology slides were retrospectively reviewed.

Results: Over a 15-year period, a total of 104 EUS-guided FNA cases were diagnosed as GIST or as a spindle cell neoplasm in which GIST was in the cytologic differential diagnosis. The patients included 57 males and 47 females with a mean age of 65 and an age range of 21 to 93 years. Gastric GISTs accounted for 75 of the 104 cases (72%). The tumors ranged in size from 0.6 to 15.0 cm (mean: 4 cm). The cytomorphologic diagnosis of GIST was supported by positive c-kit immunostaining performed on either cell block sections or follow-up surgical biopsy in 57 patients (55%). Two cases that were negative for c-kit had no additional follow-up. Histologic follow-up was available for 50 patients (48%). Follow-up histologic diagnoses other than GIST were found in 6 cases: leiomyosarcoma (1 case), leiomyoma (2 cases), synovial sarcoma (1 case), neurofibroma (2 cases). Of 48 patients with available clinical follow-up 12 (25%) were found to have developed metastases.

Conclusions: EUS-guided FNA, along with confirmatory c-kit immunostaining, is an accurate method of establishing a preoperative pathologic diagnosis of GIST. In our study, all subclassification errors occurred in cases in which confirmatory immunostaining could not be performed due to hypocellularity of the sample.

630 Ribonucleotide Reductase M2 Subunit Is a Novel Marker and a Potential Therapeutic Target for Gastric Carcinoma

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Background: Ribonucleotide reductase M2 subunit (RRM2) is essential for the production of deoxyribonucleotides before DNA synthesis in dividing cells. In some cancer types, *RRM2* mRNA expression level correlates with chemoresistance and poor patient outcome. However, there is no information concerning protein expression and the function of RRM2 in gastric carcinoma (GC).

Design: One hundred and fourteen GCs were immunohistochemically analyzed for RRM2 protein expression using a monoclonal antibody for RRM2 and a tissue microarray. The correlations between RRM2 expression and clinicopathological factors were statistically analyzed. To investigate the roles of RRM2 in GC, three GC cell lines, MKN-1, MKN-7, and SNU-719 (derived from poorly differentiated-, well differentiated-, and Epstein-Barr virus-associated GC, respectively) were transfected with RRM2 small interfering RNA and the effects on cell growth were investigated by MTT assay.

Results: RRM2 protein expression was detected in 52 of 114 GCs (45.6%), whereas it was absent in normal gastric foveolar epithelium. RRM2 expression was positively correlated with muscularis propria invasion (p=0.0002), venous invasion (p=0.0012), lymphatic invasion (p=0.034), and lymph node metastasis (p=0.037). Knockdown of the RRM2 expression by small interfering RNA resulted in decreased cell growth in all three GC cell lines investigated.

Conclusions: We demonstrated that RRM2 protein expression was correlated with the malignant potential of GC and that RRM2 had an important role in GC cell growth. Taken together, RRM2 is a novel diagnostic/prognostic marker and a potential therapeutic target molecule for GC.

631 Ten Year History of Colorectal Secondary Tumors: Hôpital St-Antoine, and Gustave-Roussy Institut Experience

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Background: Unlike the small bowel, where secondary tumors are as common as the primary ones, the colorectal mucosa is seldom the site of metastatic disease. Metastases from breast or lung carcinomas are the most common in females, whereas gastrointestinal tract and lung tumors are the most common source of metastatic lesions in males. We report here 22 cases of truly colorectal metastases from two institutions, widely involved in the treatment of colorectal tumors, during the last decade.

Design: During the last decade, 4912 patients underwent colorectal surgery for malignant tumors, of which 87 for secondary tumors. Sixty five cases were excluded, either because the involvement of colon and rectum was by direct invasion from neighbouring tumors, or because we could not rule out the extension from pertioneal or pelvic seeding, even after a long follow-up (stomach:5 cases, pancreas:1, ovary:25, uterus, including cervix and vagina: 26, prostate:2, urinary bladder:4, and tube:2). Only 22 cases (3 men; mean age, 59 years) were included. The mean disease free intreval was 6 years (range:1month-18years). At the time of follow-up, 17 patients died from disease, with a mean survival time of 9 months (range:1-21 months). Only one patient is still alive (6 months) and the remaining 4 were lost to follow-up.

Results: The patients presented with obstruction, bleeding or abdominal pain. The sources of metastases were : breast (11 cases), Melanoma (6: 1 Choroid, 1 Anus, and 4 skin), Sarcoma (3: 1 osteosarcoma, 2 leyomyosarcoma), pancreas (1), and kidney (1). The metastases were located in the right colon (8 cases), left colon (8), transverse colon (4), rectum (1), diffuse (1). Grossly, the most common feature was polypoid lesion, nodule, ulceration or diffuse thickening wall were less common. Microscopic faetures were almost similar to the primary tumors. Immunohistochemistry were performed on the biopsy specimen, mainly for breast metastases, and melanoma.

Conclusions: Truly colorectal metastases are exceptional, they represent only 0.002% of colorectal tumors in our series.They reflect a poor prognosis and any therapy is usually palliative. Therefore, the pathologist should be alert for the possibility of secondary tumors when studying large bowel biopsies, in which the appearences of the tumor are unusual or unlike ordinary colorectal cancer.

632 Leptin Expression Correlates with Favorable Clinicopathologic Phenotype and Better Prognosis in Colorectal Adenocarcinoma

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Background: Leptin, the product of the *ob* gene, is an adipocyte-derived neurohormone that regulates body fat storage and feeding behavior. Some studies have suggested that leptin has growth factor-like functions in epithelial cells and its abnormal expression may be involved in cancer development and progression. We investigated the leptin expression in normal and neoplastic colorectal tissues and their association with the clinicopathological features and clinical outcome in colorectal adenocarcinoma patients.

Design: Leptin expression was evaluated on the tissue microarray of 44 normal colon mucosal tissues, 44 adenomatous polyps and 437 colorectal adenocarcinomas by immunohistochemistry. Data was analyzed by chi-square test, one-way ANOVA, Cox regression hazards model and log-rank test with Kaplan-Meier curves.

Results: Frequency of leptin expression was dramatically increased from normal colonic mucosa (2/44, 4.5%) to adenomas (13/44, 29.5%) and adenocarcinomas (321/437, 73.5%) as neoplastic progression. Interestingly, leptin expression was correlated with favorable tumor features in depth of invasion ($p = 0.002$), lymph node metastasis ($p = 0.041$), AJCC and Dukes' stage ($p = 0.005$ and $p = 0.002$, respectively), differentiation ($p = 0.002$) and lymphatic invasion ($p = 0.010$). In univariate survival analysis, patients with leptin positive adenocarcinoma revealed better overall and disease-free survivals ($p = 0.032$ and $p = 0.004$, respectively, log-rank test). In multivariate survival analysis with the Cox proportional hazards model, leptin expression was an independent prognostic marker of disease-free survival ($p = 0.009$).

Conclusions: Leptin was gradually expressed during the normal-adenoma-adenocarcinoma sequence, suggesting an association in colorectal carcinogenesis. In addition, high leptin expression was an indicator of favorable tumor features and better survival of colorectal cancer patients.

633 Comparison between Biopsy-Based Diagnosis and Endoscopic Submucosal Dissection-Based Diagnosis of the Same Lesion in the Esophagus: Its Pitfall in a Diagnosis of Low-Grade Squamous Intraepithelial Neoplasia

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Background: It has been noted that there are significant differences between Japanese and Western criteria for squamous cell carcinoma of the esophagus. Japanese pathologist mainly focus on nuclear and structural findings, whereas Western pathologists pay attention to invasion of the dysplastic cells into the lamina propria. Here, we compared and evaluated the biopsy-based diagnoses and endoscopic submucosal dissection (ESD)-based diagnoses of the same lesion in the esophagus by using both Japanese and Western criteria.

Design: We reviewed a total of 15 ESD specimens as well as the biopsy specimens of the same lesion. The ESD specimens were given the following pathologic diagnosis used in Japan: 1) squamous cell carcinoma (M1: intraepithelial non-invasive carcinoma); 3 cases, 2) squamous cell carcinoma (M2: carcinoma involving the lamina propria); 10 cases, and 3) squamous cell carcinoma (M3: carcinoma extending to, or invading the muscularis mucosa); 2 cases. The biopsy specimens were reevaluated by using Japanese and Western criteria, respectively.

Results: The biopsy specimens of the same lesions revealed noninvasive carcinoma, namely high-grade intraepithelial neoplasia in all 15 cases based on Japanese criteria, while 8 cases were diagnosed as low-grade intraepithelial neoplasia and 7 cases were as high-grade intraepithelial neoplasia by Western criteria. Two out of eight cases with low-grade intraepithelial neoplasia by Western criteria turned out to be squamous cell carcinoma (M3), suggesting that there may be a low-grade intraepithelial neoplasia-like noninvasive carcinoma, namely basal layer type squamous cell carcinoma in situ present.

Conclusions: It should be noted that early invasive carcinoma of the esophagus is not always associated with total layer type squamous cell carcinoma *in situ*, and may be derived from basal layer type squamous cell carcinoma *in situ*. Therefore, it should be cautions to make a diagnosis of low-grade intraepithelial neoplasia when the nuclear atypia is conspicuous. Such an underdiagnosis may lead to delayed treatment.

634 Generation and Initial Characterization of a c-KIT Cre-ERT2 Mouse Model

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Background: The receptor tyrosine kinase c-KIT is physiologically expressed in embryonic and haematopoetic stem cells, eosinophilic granulocytes, melanocytes and interstitial cells of Cajal (ICCs). c-KIT is essential in hematopoiesis and germ cell evolution. In the GI tract, only mast cells and ICCs express the c-KIT receptor. The ICCs are responsible for generating slow wave motility. They are in close vicinity to the smooth muscle cells of the lamina muscularis propria and to the inhibitory and excitatory neurons. Mice with a diminished number of ICCs show a reduced NO dependent neurotransmission. This observation and their close vicinity to the enteric neurons and smooth muscle cells indicate that the ICCs are important for signal transduction in the GI tract.

Design: To obtain more information about the role and function of the ICCs, we generated a mouse model that expresses a tamoxifen activated cre-recombinase under the control of the c-KIT promoter (c-KIT Cre-ERT2). Thus a tissue and cell specific deletion of relevant genes (e.g. cGMP-dependent protein kinase type I (cGKI) known as downstream target of the NO signalling pathway) in c-KIT positive cells can be achieved. Because conventional c-KIT knock out mice (c-KIT W/W) die within the first days after birth, they are off limited usefulness for the investigation of ICCs. In our model specific genes can be influenced in a time and tissue specific manner to get more information about the role of ICCs in vivo.

Results: Here we show the successful tissue specific expression of the tamoxifen activating Cre-recombinase under the control of the c-KIT promoter using the pCAGGS-Promoter- lox-Lac-Z 3xpA-lox EGFP (Z/EG) reporter gene mouse model and the Rosa26+/LacZ reporter gene mouse model.

Conclusions: To prove that a cell specific cre-recombinase expression in the nucleus of c-KIT positive cells is present c-Kit Cre-ERT2 mice were crossbred with Z/EG reporter gene mice and the Rosa26+/LacZ reporter gene mice. After tamoxifen induced activation of the cre-recombinase a cell specific reporter-gene expression could be detected by confocal laser microscopy, confocal endo-microscopy and conventional light microscopy. Thus, this animal model established at the Department of Internal Medicine II of the Technical University of Munich is suitable for further studies of the role of ICCs in GI-motility and development of GISTs. To our knowledge this is the first mouse model expressing a tamoxifen activating cre-recombinase in ICCs.

635 Focal Duodenal Intraepithelial Lymphocytosis and Underlying Lymphoid Aggregates: The Utility of Deeper Histologic Sections

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Background: The diagnosis of isolated duodenal intraepithelial lymphocytosis is being increasingly rendered. Although it may be seen in celiac disease, this finding is non-specific. This study was performed to determine if focal increases in intraepithelial lymphocytes (IELs) may be associated with underlying lymphoid aggregates.

Design: Thirty-eight cases were identified with focal duodenal intraepithelial lymphocytosis, defined as greater than 30 IELs/100 enterocytes in 3 contiguous villi with normal findings in the remainder of the biopsy. Biopsies in which increased IELs were diffusely present were excluded from the analysis. One surface recut and five levels were then obtained for each biopsy and the presence of lymphoid aggregates or other histologic findings were noted. Indications for biopsy, final pathologic diagnosis, associated comorbidities, and documented serologic and histologic evidence of known causes of increased duodenal IELs were obtained from the medical record.

Results: Thirty-eight duodenal biopsies were reviewed in 38 patients ranging from 24 to 88 years of age (mean 51 years; 74% female) with focal intraepithelial lymphocytosis (mean of 41 ± 7.4 IELs). In deeper histologic sections, lymphoid aggregates were present in 19 biopsies (50%); 14 of these biopsies showed that the lymphoid aggregate(s) were either directly subjacent or within 3 villi of the focus of increased IELs. In the remaining 5 biopsies, the exact relationship of the lymphoid aggregate to the focus of increased IELs was not evaluable. A history of celiac disease was noted in 2 patients in the non-lymphoid aggregate group; whereas no patients with celiac disease were seen in the lymphoid aggregate group. Otherwise, there were no statistical differences in associated medical conditions and serologic status between the two groups.

Conclusions: Focal duodenal intraepithelial lymphocytosis is a non-specific finding. Half of the cases were found to be non-pathologic, as the focus of intraepithelial lymphocytosis was related to an adjacent lymphoid aggregate. Thus, multiple step sections are needed to exclude a normal finding.

636 Mycophenolate Mofetil (Cellcept®) Induced Injury of the Upper GI-Tract

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Background: Mycophenolate Mofetil (MM) is an immunosuppressant drug commonly used in patients undergoing solid organ transplant. While its pattern of inducing injury in the colon is well- known and features prominent crypt apoptosis that mimics graft versus-host-disease (GVHD), the injury pattern in the upper gastrointestinal (GI)-tract is less extensively documented. We studied the pattern of upper GI-tract injury patterns in patients taking MM.

Design: Seventeen solid organ transplant patients who were taking MM and had concurrent upper GI-tract biopsies were identified on a laboratory information system search. From these 17 patients, 15 duodenal, 15 gastric, and 6 esophageal biopsies were examined. Apoptosis and patterns of chronic injuries were assessed on standard H&E stained slides. In order to measure the significance of apoptosis, we standardized the apoptotic counts in normal biopsies using 26 normal control cases and performed statistical analysis. For the purposes of this study, we regarded apoptotic counts higher than the mean plus two standard deviations as significant. Thus the cut off values for apoptosis were >9 apoptotic bodies /100 crypts for duodenum, and >3/10HPF for both stomach and esophagus.

Results: Upper GI-symptoms manifested between 1 months to 10 years post-transplant and included nausea, vomiting, abdominal pain, odynophagia, dyspepsia, dysphagia, and GI-bleeding. Most (11/15, 73%) duodenal biopsies showed apoptotic counts of > 9/100 crypts; 67% (10/15) of gastric biopsies showed apoptotic counts of >3/10HPF, and all esophageal biopsies (6/6) showed apoptotic counts of >3/10 HPF. Two gastric biopsies with the highest apoptotic counts also showed a previously undescribed injury pattern of parietal cells resembling ballooning degeneration. Additional pathological findings included: intraepithelial lymphocytosis, chronic peptic duodenitis, and Brunner gland hyperplasia in duodenal biopsies; active and inactive chronic gastritis, and chemical gastropathy. *H. pylori* organisms were absent in gastric biopsies; ulcers seen in half (3/6) of esophageal biopsies. In most of the patients, symptoms were improved upon withdrawing MM or decreasing dosage (8/9).

Conclusions: As noted by others (Parfitt JR, Jayakumar S, Driman DK. Am J Surg Pathol 2008 32(9):1367-72.), MM-associated injury at the upper GI tract, like that in the colon, is characterized by prominent apoptosis similar to that of mild or grade I

GVHD injury pattern. We have established apoptotic count guidelines that we hope will facilitate recognition of MM-associated injury in the upper GI-tract.

637 Soluble Epoxide Hydrolase: A Novel Biomarker and Therapeutic Target of Ulcerative Colitis and Colitis-Induced Carcinogenesis

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Background: Cytochrome p450 mediated epoxyeicosatrienoic acids(EETs) show strong anti-inflammatory activity and are deactivated by the enzyme soluble epoxide hydrolase(sEH) by conversion to DHETs. sEH may play an important role in inflammatory diseases such as ulcerative colitis (UC), and targeting sEH could be a novel approach for decreasing inflammation. In the present study, we determine the expression pattern of sEH in UC, UC-induced dysplasia and carcinoma, and the effect of an sEH inhibitor on inflammation in a mouse colitis model.

Design: Tissue microarrays of 180 patients with UC(n=72), colitis-induced dysplasia(n=54), and colitis-induced carcinoma(n=54) were established. Adjacent normal colonic tissue was also identified in these patients(n=79). Avidin-biotin-peroxidase approach was used with anti-sEH antibody and proper +/- controls. sEH staining positivity was evaluated for its intensity on a scale of 0-3+ and compared with positive controls(liver & kidney). The effects of the sEH inhibitor AUDA-nBE on spontaneous colitis development in IL-10 KO mice were determined. AUDA-nBE was administered intramuscularly(10 and 20 mg/kg/day). Inflammatory activity and the incidence and number of ulcer formation in the colon was analyzed histopathologically and immunohistochemically.

Results: sEH showed distinct expression in the microarray samples. Normal colon displayed positivity in 39.1% (n=79), mainly in the focally reactive epithelia. In UC, 74.6%(n=72) displayed positivity, extensively expressed in the hyperplastic epithelia. 88.2%(n=54) of colitis-induced dysplasia and 93.9%(n=54) of colitis-induced carcinoma displayed positivity. Markedly increased sEH staining intensity was observed in UC, UC-induced dysplasia and carcinoma and the average staining intensity of sEH was 0.43 in normal glands, 0.89 in UC, 1.29 in dysplasia, and 1.31 in carcinoma. In the colitis mouse model, IL-10 KO control mice (n=10) formed 2.3 +/-2.1 ulcers with 75% ulcer incidence. The AUDA-nBE treated mice (n=10), formed 0.7+/-0.9 ulcers for low dose and 0.4 +/- 0.7 ulcers for high dose ($p<0.01$), with 50% and 30% ulcer incidence, respectively ($p<0.05$). Myeloperoxidase-labeled inflammatory cells were also significantly decreased in the AUDA-nBE group.

Conclusions: Our results indicate that sEH may play an important role in UC and UC-induced carcinogenesis, and sEH inhibition leads to a significant decrease in ulcer formation and inflammatory activity in UC.

638 JC Virus T-Antigen Is Associated with p53 Expression, Chromosomal Instability and LINE-1 Hypomethylation in Colorectal Cancer

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Background: JC virus has a gene encoding JC virus T-antigen (JCVT). JCVT may inactivate wild-type p53, cause chromosomal instability (CIN), and stabilize b-catenin. JCVT has been implicated in carcinogenesis in a number of human organs. A link between JCVT and the CpG island methylator phenotype (CIMP) has been suggested in colorectal cancer. However, no study has comprehensively examined the relations of JCVT with various molecular alterations and clinical outcome in a large number of colorectal cancers.

Design: We detected JCVT expression (by immunohistochemistry) in 271 (35%) of 766 colorectal cancers. Using MethyLight, we quantified DNA methylation in 8 CIMP-specific promoters (*CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3, SOCS1*) and 8 other CpG islands. We examined loss of heterozygosity (LOH) in 2p, 5q, 17q and 18q, and measured LINE-1 methylation by Pyrosequencing. Multivariate logistic regression analysis assessed independent association of each variable with JCVT. Cox regression models computed hazard ratio for cancer-specific and overall mortalities according to JCVT status, adjusted for patient and other molecular characteristics.

Results: In univariate analysis, JCVT was significantly associated with p53 expression ($p<0.0001$), p21 loss ($p<0.0001$), CIN ($p<0.0001$), nuclear b-catenin ($p=0.006$), LINE-1 hypomethylation ($p=0.002$), and inversely with CIMP ($p=0.0005$) and MSI ($p<0.0001$). In multivariate logistic regression analysis, p53+ [adjusted odds ratio (OR)=8.45; 95% confidence interval (CI), 5.72-12.5; $p<0.0001$] and CIN (adjusted OR=2.53; 95% CI, 1.38-4.62; $p=0.003$) remained highly significant. Cyclin D1 expression (adjusted OR=1.57; $p=0.02$), LINE-1 hypomethylation (adjusted OR=1.97 for a 30% decline as a unit; $p=0.03$), BRAF mutation (adjusted OR=2.20; $p=0.04$) and family history of colorectal cancer (adjusted OR=0.64; $p=0.04$) were also significantly associated with JCVT. In contrast, JCVT was not significantly associated with CIMP, MSI, β-catenin or patient survival in multivariate analyses.

Conclusions: JCVT expression in colorectal cancer is associated with p53 expression (surrogate of p53 mutation), CIN and LINE-1 hypomethylation, all of which are key events in colorectal carcinogenesis.

639 Development of Multilayerd Epithelium (ME) after Photodynamic Therapy (PDT): Does MLE Represent an Inevitable Progression to Barrett's Esophagus (BE)?

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Background: PDT is used to ablate BE and BE-related neoplasia, which then leads to restoration of squamous epithelium, over a variable period of time. ME is a distinctive hybrid-type epithelium with squamous and columnar features that has been observed in association with shorter lengths of BE and is believed to be a precursor to BE. However, ME has also been reported in post-PDT biopsies raising the possibility that ME may represent an intermediate step in the columnar to squamous transition post PDT as well. The aim of this study was to determine the prevalence and significance of ME in pre- and post-PDT biopsies.

Design: The study cohort consisted of 47 BE patients (mean age: 71 years, M:F = 38:9, mean BE length: 6.4 cm) with high-grade dysplasia (n=13), intramucosal adenocarcinoma (n=28) and invasive adenocarcinoma (n=6), all of whom were treated with PDT. All patients were also on proton pump inhibitor therapy. Four quadrant biopsies, every 2 cm, were taken pre and post PDT from the site of the original BE segment. The presence of ME in pre- and post-PDT biopsies was correlated with several clinicopathological features.

Results: ME was found in 4 patients (8.5%) pre PDT and in 24 (51.1%) post PDT. There was a trend towards ME observed more often post PDT (43/456 endoscopies, 9.4%) than pre PDT (4/72, 5.6%). ME was noted only in biopsy levels within 2 cm of GE junction and was often associated with esophageal mucosal glands and/or squamous islands. ME was seen in biopsies performed at an average of 15.7 months after PDT (range: 1 - 63 months). The original BE segment was longer in patients who developed ME post PDT (ME+ group) than in those who did not (ME- group) (7.54cm vs. 5.13cm, respectively, $p = 0.01$). There was no difference in other clinicopathologic features between the 2 groups. At the end of the follow-up period (mean: 48 months), neoplasia persisted in 10 patients (4 in the ME+ and 6 in the ME- groups, $p= 0.36$) and residual BE was observed in 15 patients (8 in the ME+ and 7 in the ME- groups, $P=0.59$).

Conclusions: ME often develops after PDT and is associated with longer BE segment length. The presence of post-PDT ME does not correlate with persistence of BE and/or neoplasia. The results suggest that ME can be seen during the process of squamous restitution (an intermediate step in the columnar to squamous conversion) and that ME does not always represent an inevitable progression to BE.

640 Natural History of Sporadic Gastric Dysplasia in the U.S.A: Follow up Study of 54 Patients

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Background: Our understanding of the natural history of sporadic gastric epithelial dysplasia (GED) has been shaped predominantly by Asian and European series. In these regions, GED has been shown to represent a high risk marker for undiagnosed gastric adenocarcinoma and that the histologic grade correlates with the propensity for progression into gastric cancer. To date, there are limited data regarding the natural history of GED in the U.S. where the incidence of gastric adenocarcinoma is low.

Design: Cases of consecutive non syndromic patients diagnosed with GED during a 5 1/2 year time period were accrued. All available biopsies were collected and evaluated for grade, location and histologic subtypes, i.e. type 1 (intestinal) and type 2(foveolar). Electronic clinical charts were reviewed for demographics and outcome.

Results: Fifty-four patients (M/F=29/25; mean age 66 years) were diagnosed with gastric dysplasia. The most frequent presenting symptoms were abdominal pain (33%) and anemia (16.7%). At initial endoscopy, 36 patients were diagnosed with low grade dysplasia (LGD) and 18 with high grade dysplasia(HGD). No carcinomas were detected at initial presentation. Twenty-three lesions were classified as type 1 dysplasia (42.6%) (LGD: 13,HGD:10) and 29 as type 2 dysplasia (53.7%) (LGD:23,HGD:6). Two cases reveled a mixed type 1 and 2 phenotype. The topographic distribution of type 1 and 2 GED was different between body /fundus and antrum (see table).Follow-up was available for 32 patients (mean 35.5,range 1-119 months), 34% of whom had persistent dysplasia. Regression occured in 34% of the patients, and was more common in type 2 dysplasia. Progression to early adenocarcinoma was observed in 31% of patients (n=10). None of the patients developed an advanced adenocarcinoma during the follow up.

Type, grade and progression of GED

	Median age	Antrum*	Body/Fundus	Progression	Regression*	Stable
Type 1 (n=23)	70.1	18 (78%)	5 (22%)	2	1	7
Type 2 (n=29)	59.8	5 (17%)	24 (83%)	7	10	3

* Difference in distribution and regression was significant: $p<0.01$

Conclusions: In contrast to high risks regions, patients with GED in the United States show a very low rate of concurent gastric adenocarcinoma and progression to advanced neoplasms. Our data also show that type 1 and type 2 GED demonstrate differences in topography and biologic behavior. The role of ethnic differences and the impact of modern endoscopic modalities in these results remain to be evaluated.

641 LINE-1 Hypomethylation Is an Independent Predictor of Poor Prognosis in Colon Cancer

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Background: Genome-wide DNA hypomethylation plays an important role in genomic instability and colonic carcinogenesis. However, the relation between global DNA methylation level in colon cancer and patient outcome remains uncertain.

Design: Utilizing 643 colon cancers with adequate follow-up in two independent prospective cohorts, we quantified methylation in a repetitive DNA element LINE-1 (long interspersed nucleotide element-1) by Pyrosequencing, which correlates well with global DNA methylation (5-methylcytosine) level. Kaplan-Meier analysis was performed to compare survival time distributions. Multivariate Cox proportional hazard models were used to compute hazard ratios (HRs) of colon cancer-specific and overall mortalities, adjusted for patient characteristics and tumoral molecular features,

including the CpG island methylator phenotype (CIMP), microsatellite instability (MSI), chromosomal instability (CIN), KRAS, BRAF and p53.
Results: Tumoral LINE-1 methylation levels were approximately normally distributed (mean 61.2%, median 62.1%, standard deviation 9.4%). When LINE-1 methylation was used as a continuous variable, LINE-1 hypomethylation was linearly associated with a significant increase in both colon-cancer specific mortality (P_{trend}=0.0009) and overall mortality (P_{trend}=0.002). In addition, compared to patients with ≥75% LINE-1 methylated tumors, the adjusted HRs for colon cancer-specific mortality were 1.79 [95% confidence interval (CI), 0.81-3.97] for patients with 60-75% LINE-1 methylation, 2.43 (95% CI, 1.08-5.49) for those with 45-60% LINE-1 methylation, and 5.00 (95% CI, 1.92-13.1) for those with <45% LINE-1 methylation. Similar findings were noted for overall mortality. Kaplan-Meier analysis also showed a significant difference in colon cancer-specific survival time distributions among those LINE-1 categories (P=0.0001). The influence of LINE-1 hypomethylation on survival was consistent across the two independent cohort studies as well as across strata of clinical or molecular characteristics.
Conclusions: Tumoral LINE-1 hypomethylation, which reflects genome-wide DNA hypomethylation, is an independent predictor of shorter survival among colon cancer patients.

642 *PIK3CA* Mutation Predicts Poor Prognosis in Stage I-III Colorectal Cancer

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Background: *PIK3CA* mutation and subsequent activation of the AKT pathway play an important role in the development of a subset of colorectal cancers. However, little has been known on the prognostic significance of *PIK3CA* mutation in colorectal cancer.
Design: Utilizing 751 colorectal cancers (stage I-IV) in two independent prospective cohort studies (the Nurses' Health Study and the Health Professionals Follow-up Study), we detected *PIK3CA* mutation in 117 (16%) tumors by Pyrosequencing. Cox proportional hazard models were used to compute hazard ratios (HRs) of colon cancer-specific and overall mortalities, unadjusted and adjusting for patient characteristics and tumoral molecular features, including AJCC stage, microsatellite instability (MSI), the CpG island methylator phenotype (CIMP), LINE-1 methylation, *KRAS*, *BRAF* and p53. An interaction between *PIK3CA* and *KRAS* mutations was assessed using the cross product term of the *PIK3CA* and *KRAS* variables and likelihood ratio test.
Results: Among stage I-III colorectal cancers, *PIK3CA* mutation was associated with an increase in cancer specific mortality by univariate analysis [HR 1.64; 95% confidence interval (CI), 0.95-2.86], which became significant after adjusting for other potential prognostic factors in multivariate analysis (adjusted HR 2.23, 95% CI, 1.21-4.11). The effect of *PIK3CA* mutation on mortality appeared to differ according to *KRAS*-mutational status. Among patients with *KRAS*-wild-type tumors, the presence of *PIK3CA* mutation was associated with a significant increase in cancer-specific mortality (HR 3.80, 95% CI, 1.56-9.27). In contrast, *PIK3CA* mutation conferred no significant effect on cancer-specific mortality among patients with *KRAS*-mutated tumors (HR 1.25; 95% CI, 0.52-2.96), although the interaction was not statistically significant ($P_{interaction}$=0.13). *PIK3CA* mutation was unrelated with prognosis in stage IV tumors.
Conclusions: *PIK3CA* mutation is associated with shorter cancer-specific survival in stage I-III colorectal cancer. The adverse effect of *PIK3CA* mutation may be potentially limited to patients with *KRAS*-wild-type tumors.

643 Cyclin D1 Expression Marks a Subset of Colon Cancer with Good Prognosis

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Background: Cyclin D1 and cyclin-dependent kinases (CDK), which are commonly activated in colon cancer, facilitate cell cycle progression. The activity of cyclin D1 can be blocked by CDK inhibitors, such as p27 (*CDKN1B*) and p21 (*CDKN1A*, which is induced by p53). Previous prognostic data on cyclin D1 in colon cancer have been conflicting, and no previous study has considered confounding effect of p53, p21, p27 and other related molecular events [microsatellite instability (MSI) and the CpG island methylation phenotype (CIMP)].
Design: Among 602 colon cancer patients (stage I-IV) in two independent prospective cohort studies, cyclin D1 expression was detected in 330 (55%) tumors by immunohistochemistry. Cox proportional hazard models computed hazard ratios (HRs) of colon cancer-specific and overall mortalities according to cyclin D1 status, adjusted for patient characteristics and tumoral molecular features, including p53, p21, p27, COX-2, fatty acid synthase (FASN), CIMP, MSI, *KRAS* and *BRAF*.
Results: Cyclin D1 overexpression was associated with a low cancer-specific mortality in Kaplan-Meier analysis (p=0.006), and in both univariate Cox regression [unadjusted HR 0.64; 95% confidence interval (CI), 0.47-0.88] and multivariate analyses (adjusted HR 0.57; 95% CI, 0.39-0.84). Similar findings were observed for an overall mortality. Notably, the effect of cyclin D1 on survival might differ by MSI status ($P_{interaction}$=0.008). Compared to tumors that were both cyclin D1-negative and non-MSI-high, the presence of either cyclin D1 or MSI-high or both appeared to confer better prognosis (adjusted HR point estimates 0.10-0.65). The beneficial effect of cyclin D1 expression did not significantly differ between the two independent cohort studies ($P_{interaction}$=0.19) and among other strata of patient and molecular characteristics.
Conclusions: Cyclin D1 expression predicts a low mortality in colon cancer. In order to become malignant tumors, cyclin D1-negative tumors may need to have other aberrations (bypassing cyclin D1 activation), which may lead to more aggressive tumors than cyclin D1-positive tumors. Thus, positivity for oncogene activation (associated with the absence of more deleterious aberrations) can mark a subset of colon cancer with good prognosis.

644 Identification of Histologically Unique Adenomas That Arise Predominately in Patients with CIMP-Associated Polyps

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Background: Serrated polyps have now been subdivided into 5 histologic categories some of which (sessile serrated adenomas [SSAs] and traditional serrated adenomas [TSAs]) have evidence of CpG island methylation (CIMP). However, for the most part adenomas continue to be regarded as a single entitity. We have recently shown that patients with one SSA are much more likely to harbor synchronous TSAs and SSAs, suggesting that there is a field defect in the colons of these patients that predisposes to CIMP-associated polyps. As many of these SSA patients also had apparently typical adenomas, we set out to determine if there were subtle morphologic differences that could distinquish between adenomas from SSA patients and those from non-SSA patients.
Design: A search of our pathology database revealed 105 adenomas from 71 pts. harboring at least one SSA. Seventy-nine consecutive adenomas from 36 pts. without SSAs were also identified. None had features sufficient for a diagnosis of traditional serrated adenoma or SSA with dysplasia, and all appeared as typical adenomas at first glance. Two GI pathologists blindly analyzed these polyps according to ten criteria: focal serration, crypt dilatation, cytoplasmic eosinophila, crypt branching, villous architecture, luminal debris, apoptosis, nuclear shape, type of mucin, and intraepithelial lymphocytes.
Results: Adenomas from patients with SSAs were larger (5.4 mm vs. 4mm, p<0.001) and were significantly more likely to exhibit focal serration, crypt dilatation, cytoplasmic eosinophilia, and increased apoptosis as compared to adenomas from non-SSA patients. 22 of the 105 adenomas in SSA patients demonstrated focal serration, dilation, and eosinophilic cytoplasm compared with only 2 adenomas from non-SSA patients. The location of these adenomas is not significantly different from adenomas in non-SSA patients.

Characteristic		Adenomas from SSA patients	Adenomas from Non-SSA patients	p-value
Focal serration				
	Present	30.4%	5.1%	0.00002
Dilation				
	Present	43.9%	28.2%	0.036
Debris				
	Present	20.0%	12.8%	ns
Branching				
	Present	55.2%	47.4%	ns
Villous architecture				
	Present	3.8%	7.7%	ns
Eosinophilic cytoplasm				
	+	36.1%	21.8%	0.031
	++	26.7%	1.3%	0.00001
Apoptosis				
	+	43.8%	66.7%	0.003
	++	20.1%	6.4%	0.006
Mucin				
	Microvesicular	19.0%	23.0%	ns
	Goblet Cell	31.4%	28.2%	ns
	Poor	45.7%	43.6%	ns
	Foveolar	1.9%	1.3%	ns
IELs				
	Present	1.0%	1.2%	ns

Conclusions: A subset of adenomas in SSA patients are histologically distinct from adenomas in non-SSA patients and may arise through the CIMP-pathway. Molecular analysis of these polyps will be necessary to confirm this hypothesis. These results indicate that adenomas may now be divisible into histologic subtypes based on underlying molecular differences.

645 Interobserver Agreement in rhe Diagnosis of Serrated Polyps and Identification of Prolapse Hyperplastic Polyps as Histologic Mimics of Sessile Serrated Adenomas

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Background: Sessile serrated adenomas (SSAs) can be extremely difficult to distinguish from hyperplastic polyps (HPs), particularly the microvesicular HP. In one recent study the interobserver agreement in the diagnosis of serrated lesions was moderate with a kappa score of 0.58. In that study only sessile serrated adenomas larger than 5 mm and those that were well-oriented were included. In this study we set out to determine the interobserver agreement in serrated lesions independent of polyp size and orientation. Moreover, our aim was to identify any unique characteristics of those polyps that presented the most difficulty in the histologic diagnosis.
Design: 172 SSAs, 13 SSAs with dysplasia, and 89 hyperplastic polyps were identified from our surgical pathology database. Two GI pathologists independently reviewed these polyps blinded to location. After this independent review, a consensus conference was convened to resolve any differences between the two pathologists or with the sign-out diagnoses.
Results: The interobserver agreement as measured by the Cohen's kappa score was 0.63 (moderate). At the consensus conference, 15 polyps originally diagnosed as SSAs

were designated as hyperplastic polyps. Of these, 9 were located in the left colon and had features of mucosal prolapse. 36 left sided SSAs were subsequently reviewed for evidence of prolapse. 18/36 (50%) had features of prolapse in addition to features diagnostic of an SSA.

Location	Sign-out diagnosis	JH	AN	Consensus diagnosis
Unknown	SSA	Prolapse HP	Prolapse HP	Prolapse HP
Sigmoid	SSA	HP	Prolapse HP	Prolapse HP
Rectum	SSA	Prolapse HP	HP	Prolapse HP
rectum	SSA	Prolapse HP	SSA	Prolapse HP
sigmoid	SSA	Prolapse HP	SSA	Prolapse HP
rectum	SSA	HP	SSA	Prolapse HP
sigmoid	SSA	SSA	Prolapse HP	Prolapse HP
rectum	SSA	SSA	Prolapse HP	Prolapse HP
sigmoid	SSA	SSA	Prolapse HP	Prolapse HP
hepatic flexure	SSA	HP	HP	HP
cecum	SSA	HP	SA	HP
hepatic flexure	SSA	SSA	HP	HP
ascending	SSA	SSA	HP	HP
splenic	SSA	HP	SSA	HP
rectum	SSA	SSAD	SSAD	SSAD

Conclusions: The diagnosis of serrated lesions is quite difficult as there is considerable overlap between hyperplastic polyps and SSAs. In this study, we show that the interobserver agreement in the diagnosis of serrated lesions regardless of size and orientation is moderate with a Cohen's kappa score of 0.63. We also identify prolapsed hyperplastic polyps as histologic mimics of SSAs. Mucosal prolapse has been known to cause architectural abnormalities such as crypt branching and dilation. However, 50% of SSAs in the left colon can also show features of prolapse. Thus when diagnosing a SSA in the left colon, one must be sure to exclude the possibility of a hyperplastic polyp with superimposed features of mucosal prolapse.

646 Expression of Cadherin 17 (CDH17) in the Gastrointestinal Tract, Liver and Pancreas: Comparison with CDX2 as a Diagnostic Marker

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Background: CDH17 is a member of the cadherin superfamily with expression in normal intestinal epithelium and fetal hepatocytes. CDX2 is thought to regulate the transcription of CDH17 in human colonocytes. Although CDH17 expression has been sporadically reported in carcinomas of the colon, stomach, pancreas, and liver, systematic evaluation of this marker has not yet been performed. In this study, we compared the expression of CDH17 to that of CDX2 in carcinomas and normal tissues from the esophagus, stomach, colon, pancreas, and liver, in order to determine whether the differential expression of these markers may be a useful tool in the diagnosis of gastrointestinal malignancies.

Design: We created a tissue microarray using 3 cores each of non-neoplastic and tumor tissues from 47 cases of esophageal adenocarcinoma (n=5), esophageal squamous cell carcinoma (n=4), diffuse-type (n=5) and intestinal-type (n=5) gastric carcinoma, colonic adenocarcinoma (n=10), pancreatic ductal adenocarcinoma (n=9), and hepatocellular carcinoma (n=9). Immunohistochemical stains for CDH17 and CDX2 were performed using standard techniques. Positivity was defined as membranous staining for CDH17 and nuclear staining for CDX2. Intensity and extent of staining (focal vs. diffuse) were evaluated.

Results: Benign colonic epithelium co-expressed CDH17 and CDX2, whereas normal esophageal and gastric epithelium, hepatocytes, and bile ducts were negative for both markers. Benign pancreatic ductal epithelium expressed CDH17 but showed only focal, weak CDX2 reactivity. All adenocarcinomas of the esophagus, stomach and colon were concordantly positive (22 cases) or negative (1 esophageal and 2 gastric) for CDH17 and CDX2 expression, and esophageal squamous cell carcinomas were uniformly negative for both markers. In contrast, 6 (67%) pancreatic ductal adenocarcinomas diffusely expressed CDH17, one of which also expressed CDX2. Focal CDH17 staining was noted in one hepatocellular carcinoma, but none expressed CDX2.

Conclusions: Co-expression of CDH17 and CDX2 is uniformly present in colonic adenocarcinomas, and is common in esophageal and gastric adenocarcinomas. Thus, CDH17 may be used in conjunction with CDX2 to confirm intestinal differentiation among adenocarcinomas. Importantly, most pancreatic adenocarcinomas express CDH17, but not CDX2, indicating that isolated CDH17 expression may be useful in identifying carcinomas of the pancreas.

647 Sessile Serrated Adenomas of the Colon Are Associated with Methylation Induced Loss of Cdx2 Expression

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Background: Sessile serrated adenomas (SSAs) are generally characterized by histologic features of gastric mucosa, the immunophenotypic gain of expression of gastric type markers such as Sox2, Muc5A and Muc6, and loss of expression of the intestine-specific transcription factor Cdx2. Our goal was to determine the methylation status of Cdx2 and correlate this parameter to Cdx2 expression in serrated polyps of the colorectum with and without neoplastic potential.

Design: We obtained paraffin-embedded samples of hyperplastic polyps (HP) and sessile serrated adenomas (SSA) 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 Cdx2 was performed using standard methods, and scored for the presence and absence of nuclear labeling as well as the distribution of positive labeling in each polyp. DNA was also extracted from each polyp, bisulfite treated and used for methylation specific PCR (MSP) of the CDX2 promoter. Distributions were compared using the Student's T-test and frequencies were compared using the Chi-squared test, or the Fisher exact test for sample sizes <5. p-values ≤0.05 were considered significant.

Results: A total of 31 SSAs (19 with and 12 without associated low grade dysplasia, LGD) and 17 HPs from 48 patients were studied. There was no significant difference in age (57.5±9.5yrs vs 61.6±9.5yrs) or gender (8F:7M vs 16M:16F) among patients with HP versus SSA. However, SSAs were more frequently located in the right/transverse colon (29/31) compared to HPs (1/18, p=0.0001). MSP analyses indicated promoter hypermethylation of the CDX2 promoter in 20/48 polyps (42%). When promoter methylation was compared to polyp histology, the frequency of CDX2 methylation was 3/18 HPs (17%), 4/12 SSAs (25%) and 14/19 SSAs with associated LGD (74%) (p=0.003). CDX2 promoter methylation was associated with loss of Cdx2 labeling in the same serrated polyp. However, Cdx2 labeling was also lost in serrated polyps with normal promoter methylation, suggesting additional mechanisms underlie loss of Cdx2 expression.

Conclusions: Loss of Cdx2 expression is frequent in SSAs and in some polyps may be due to CDX2 promoter hypermethylation. Cdx2 loss may contribute to the initiation or progression of sessile serrated adenomas to colorectal adenocarcinoma.

648 DNA Methylation Changes in Multistep Gastric Carcinogenesis and Their Relationship with *H. pylori* Infection and Association of Gastric Cancer

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Background: CpG island hypermethylation and genomic DNA hypomethylation are found not only in gastric cancer but also in its premalignant lesions. Helicobacter pylori infection induces aberrant CpG island hypermethylation in gastric mucosae. However, little is known about the relationship between H. pylori infection and aberrant methylation changes in premalignant lesions. The present study aimed to characterize methylation changes of a subset of genes and repetitive DNA elements and their relationship with H. pylori infection in premalignant lesions of gastric cancer.

Design: We performed MethyLight analysis of 25 genes and SAT2 and COBRA analysis of LINE-1 and ALU in a total of 200 gastric tissue samples. *H. pylori* infection was closely associated with enhanced hypermethylation of CpG island loci in chronic gastritis but this association was not found in intestinal metaplasia, gastric adenoma, and gastric cancer.

Results: *H. pylori* infection was closely associated with enhanced hypermethylation of CpG island loci in chronic gastritis but this association was not found in intestinal metaplasia, gastric adenoma, and gastric cancer. Intestinal metaplasia and gastric adenoma showed higher number of methylated genes than chronic gastritis regardless of H. pylori infection. Different methylation behaviours depending on types of genes or repetitive DNA elements were found along multistep gastric carcinogenesis. No difference was noted in the number of methylated genes in chronic gastritis or intestinal metaplasia between gastric cancer patients and non-cancer subjects.

Conclusions: Our findings suggest that CpG island hypermethylation and repetitive DNA hypomethylation tend to be enhanced with progression of the lesion along multistep gastric carcinogenesis although the timing of hypermethylation and hypomethylation is different depending on types of genes or repetitive DNA elements.

649 Modified Marsh Grade 2 Change in Duodenal Biopsies Is as Suggestive of Serological Positivity in Celiac Disease as Are Higher Grades

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Background: The diagnostic algorithm of celiac disease or gluten-sensitive enteropathy, a chronic immune-mediated enteropathy due to gluten intolerance, is complex and based upon serological and histological concordance. Antibodies against tissue transglutaminase (TTG) and endomysium (EMA) have the highest specificity and sensitivity in comparison with other serological tests. Proximal small intestinal biopsies are graded according to the modified Marsh criteria, adopted by the National Institite of Health. When intestinal biopsies display greater than Marsh 2 changes (variable villous shortening or Marsh 3), diagnosis of celiac disease is made with seropositivity. However, when serological studies are normal, celiac disease may still be diagnosed clinically by HLA studies (presence of DQ2/DQ8). Since data correlating histology with serology is limited, we correlated modified Marsh grading of duodenal biopsies with serological positivity and HLA positivity when serology is negative.

Design: Review of clinical and pathology databases from our medical center from 2004 to 2008 identified 135 adult patients clinically suspected of celiac disease who had duodenal biopsies. The following characteristics were reviewed: modified Marsh grading of the duodenal biopsies (0 = normal, 1 = IELs only, 2 = IELs + crypt proliferation, 3 a,b,c = 2 + mild, moderate and severe shortening of villi), serological positivity (TTG and EMA), and when serology was negative, presence of HLA-DQ2 or HLA-DQ8.

Results:

Correlation between Marsh grade of duodenal biopsy, serology, and HLA status.

	Marsh 0	Marsh 1	Marsh 2	Marsh 3
Patients	67/135 (50%)	7/135 (5%)	14/135 (10%)	47/135 (35%)
Serology		0/7 (0%)	14/14 (100%)	38/47 (81%)
HLA		2/5 (40%)	N/A	6/9 (67%)

Conclusions: In our experience, lower grade (modified Marsh 2) histological changes are just as predictive of celiac disease as higher grades with villous shortening. This

makes further subclassifications in modified Marsh grades 3a, 3b and 3c, less clinically useful than the recommendations currently adopted. Furthermore, our study reduces the stringency involved in the orientation of the duodenal biopsies to evaluate the shortening and puts greater emphasis in evaluating intraepithelial lymphocytes (IELs), chronic duodenitis and crypt proliferative changes.

650 Tissue Transglutaminase II Is a Key Enzyme Involved in Epithelial Regeneration and Carcinogenesis in the Setting of Ulcerative Colitis

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Background: Tissue transglutaminase II (TG2) is a unique member of transglutaminase family of enzymes that catalyze calcium-dependent posttranslational modification as well as calcium-independent activities such as hydrolysis of GTP/ATP and protein disulfide isomerase reaction. TG2 overexpression has been observed in melanoma, breast and pancreatic cancer where it contributes to cancer cell adhesion, invasion and development of chemoresistance. In this study we evaluated immunohistochemical expression of TG2 in ulcerative colitis (UC) in both humans and animal model, and UC associated dysplasia and carcinoma in humans.

Design: Colectomy specimens (n=24) with active UC, 10 cases of dysplasia (8-low grade and 2 high grade) and 13 cases of carcinoma arising in the setting of UC were used in this study. Immunohistochemical expression of TG2 was scored on a semiquantitative scale based on intensity and percentage positive cells, using appropriate controls. Dextran sulfate sodium (DSS) induced UC in mice and intestinal epithelial cell line IE6 were used to test the effect of prostaglandin E2 (PGE2) and COX-2 inhibitor, celecoxib, on TG2.

Results: Low cytoplasmic expression of TG2, predominantly localized to the regenerating epithelium adjacent to ulcers and subepithelial stromal cells, was noted in 20/24(83%) cases. Low and high grade dysplastic lesions either showed no or very low expression of TG2. In contrast, 7/13 (54%) carcinomas showed significantly high level of TG2 expression compared to regenerating/dysplastic epithelium (p=0.02). TG2 expression was also noted in regenerating epithelium in DSS induced UC in mice. Large ulcers induced by celecoxib showed decreased TG2 expression in regenerating epithelium. Using western blot approach, we found that with PGE2 treatment, IE6 cell line showed dose and time dependent induction of TG2.

Conclusions: Our findings show differential strong expression of TG2 in UC-associated carcinoma, suggesting that TG2 serves as a useful marker to differentiate carcinoma from reactive and dysplastic changes. Lack of TG2 in dysplasia indicates that overexpression of this protein is a late event in UC-associated carcinogenesis. Animal model further confirms this observation and identifies PGE2 as a regulator of TG2 expression.(Partly supported by NIH CA104741).

651 Role of Antigen Presenting Cells in the Pathogenesis of Adult Eosinophilic Esophagitis – A Single Institutional Study of 285 Patients

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Background: Although primarily idiopathic in nature, adult eosinophilic esophagitis (EE) is a distinct clinicopathologic entity known to be associated with ingested allergens, reflux, drugs, or infectious agents. Evidence indicates that immune dysregulation is the main pathogenetic mechanism and mucosal CD1a antigen presenting cells (APC) are part of this pathway. In this study, we analyzed the role of APC in pathogenesis of adult EE and correlated the findings with histological classification and diet elimination trial data.

Design: Our aim was to a) evaluate density of CD1a cells in EE, b) compare the density between distal-predominant EE and reflux esophagitis (RE), & c) measure APC response to diet elimination therapy in a 10 patient cohort non-responsive to PPI Rx (ongoing trial). The diagnostic criteria for EE were: at least 1 biopsy fragment with >25 eos/hpf or 15 eos/hpf in 3 consecutive fields. Age, biopsy location, basal cell hyperplasia (BCH), spongiosis and max.no.of eos./hpf were noted. CD1a+APC were determined using avidin-biotin-peroxidase immunohistochemistry with appropriate controls and expressed as max. no. of CD1a+ cells/hpf.

Results: We reviewed 424 biopsies from 285 patients (193 males, 92 females) over a 5-yr period. There were 97 cases of distal-predominant EE while 188 patients showed both proximal and distal esophageal involvement (diffuse pattern EE). CD1a cells were mainly located in the peri-papillary area. There was a significant difference between max. no.of CD1a +cells/hpf in normal mucosa (7.25±3.1) and RE (16.2±4.6, p=0.01) and EE (34.4±17.8, p=0.003). There was no association between density of APC and eosinophilic infiltrate, BCH or spongiosis. When compared to diffuse pattern EE (30±17), a significantly more CD1a+ cells were seen in distal-predominant EE (40±17.5, p=0.03). Similarly, higher number of CD1a+ cells were noted in distal-predominant EE compared to RE (p=0.006). Although the max. no.of eos./hpf significantly improved post diet elimination (p=0.03), no such difference was found with density of APC.

Conclusions: Our results indicate that APC play a key role in pathogenesis of EE. The CD1a cell distribution pattern suggests that process of antigen presentation is distal predominant and CD1a may serve as a useful marker to distinguish EE and RE. Although diet elimination trial does not alter the number of CD1a cells, further study of functional APC distribution is warranted.

652 Defining Crohn's Disease by Immunohisotchemistry and Histology: The Role of Metalloproteinase 3 and Its Inhibitor in Isolated Chronic Ileitis

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Background: The pathogenesis behind Crohn's disease is mutifactorial. Contributing aspects include an imbalanced inflammatory process, disorders of mucosal repair, genetic susceptibility, and bacterial infection. The extracellular matrix in the lamina propria of the gastrointestinal tract maintains the normal mucosal structure. The family of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are responsible for degrading the extracellular matrix. Many of the MMPs and TIMPs have been found to be expressed in ulcerated areas of Crohn's disease. Clinically, it is important to differentiate isolated ileitis caused by Crohn's disease from ileitis due to other causes, especially ileitis due to non-steroid anti-inflammatory drugs. In this study, we will determine if immunostaining with MMP3 and TIMP1 can help differentiate ileitis of Crohn's disease from ileitis due to other causes.

Design: Forty-six consecutive cases of isolated ileitis of the terminal ileum were retrieved. 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 accompanying chronic colitis. The diagnostic criteria of chronic ileitis included crypt distortion, plasmacytosis in lamina propria, ulceration, and/or pyloric gland metaplasia. Clinical follow-up was up to 3.5 years.

Results: Among these 46 patients, 54% (25/46) of the patients had confirmed Crohn's disease during 3.5 years follow-up. By immunohistochemistry, 88% and 88% of those ileitis caused by Crohn's disease showed expressions of MMP3 and TIMP1,respectively, comparing to 24% and 19% in those ileitis caused by non Crohn's disease.

Table 1: The Expressions of MMP3 and TIMP1 in Ileitis

	MMP3+, TIMP1+	MMP3+, TIMP1-	MMP3-, TIMP1+	MMP3+, TIMP1-	Total
Crohn's	22	2	2	1	25
nonCrohn's	3	2	1	15	21
Total	25	4	3	16	46

Conclusions: Matrix metalloproteinase 3 (MMP3) and its tissue inhibitor (TIMP1) are expressed in isolated ileitis caused by Crohn's disease, with a sensitivity of 96% and specificity of 64% if either MMP3 or its inhibitor (TIMP1) is expressed, while the sensitivity will be decreased to 88% and specificity will be increased to 86% if both MMP3 and TIMP1 expressed. The utilization of immunostains of MMP3 and TIMP1 on isolated ileitis could help early diagnosis of Crohn's disease.

653 Sessile Serrated Adenoma: Do Clinicians Understand the Concept and Manage Patients Appropriately?

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Background: The diagnosis of sessile serrated adenoma (SSA) lacks interobserver reproducibility and alternate terminologies, e.g. sessile serrated polyp (SSP), are also used to describe the same lesion. The level of awareness of this evolving concept amongst gastroenterologists and the clinical impact of rendering a diagnosis of SSA has not been evaluated. We analyzed awareness about SSA among endoscopists and tried to determine whether there is uniformity in management following a diagnosis of a SSA.

Design: An online questionnaire link was sent to practicing gastroenterologists from diverse practice settings across the US. The questions focused on the frequency of SSA diagnosis, terminology, distinction of SSA from conventional adenomas, molecular profile of SSA, diagnostic criteria for hyperplastic polyposis, and surveillance practice for patients diagnosed with SSA.

Results: 52 Gastroenterologists from 11 states participated (university:84.6%; group practice:13.5% and community:1.9%). Reported SSA prevalence varied widely from <1% (21.2% participants) to more than 10% (9.6% participants). Many gastroenterologists (40.4%) are unaware that SSP and SSA are the same lesion. Only 50% receive a diagnosis of SSA accompanied by an explanatory note. Interestingly, 75% clinicians thought that SSAs show "adenomatous" change and are variants of tubular (61.5%) or villous (11.5%) adenomas. 17.3% reported rectosigmoid or left colon as the commonest location for SSA. Almost all participants (98.1%) were aware that SSA are premalignant. Reported surveillance interval for SSA was either similar to (73.1%) or shorter than (25%) that for conventional adenomas. Most endoscopists (75%) do not seek a second opinion for a large lesion (>1.0cm) diagnosed as hyperplastic polyp. Only 51.9% were aware that colon cancer arising in SSA is genetically distinct from the usual adenoma-carcinoma progression, and only 30% knew of the association of BRAF mutation and CIMP with SSA.

Conclusions: Frequency of SSA diagnosis varies across hospitals. Conceptual understanding of this lesion is limited amongst gastroenterologists. The use of "adenoma" in SSA successfully conveys the premalignant potential to clinicians but causes confusion with tubular, villous and serrated adenoma. Low diagnostic threshold for SSA can lead to unnecessary surveillance and increased cost of care. Pathologists need to develop uniform definitions for SSA and increase awareness of this entity among their gastroenterologists.

654 Decrease in Expression of BRMS1 Correlates with the Tumor Progression and Metastatic Potential of Colorectal Adenocarcinoma

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Background: Breast Cancer Metastasis Suppressor 1 (BRMS1), a recently discovered metastasis suppressor gene, has been found to suppress multiple organ metastasis by affecting numerous steps of the metastatic cascade, without affecting primary tumor growth. Studies using human tissue have demonstrated decreased BRMS1 levels in malignant melanoma and breast cancer. However, the expression pattern of BRMS1

in primary and metastatic colorectal carcinoma has not been tested. The current study, the first of its kind, explores BRMS1 expression patterns in matched normal colonic tissue, primary colorectal carcinomas, and lymph node metastasis to explore its role in human colorectal carcinoma progression and metastasis.
Design: A tissue array containing 25 cases of primary colorectal carcinoma (19 colon and 6 rectal) with matched lymph node metastasis (22 cases) and adjacent normal colonic mucosa was utilized for the study. Immunohistochemical staining for BRMS1 was performed using a custom made monoclonal antibody (Clone 3a1.21) on paraffin-embedded formalin-fixed tissue. Expression was graded based on intensity (0-5) in the cells staining for BRMS1.
Results: BRMS1 staining was predominantly nuclear, with a diffuse and intense staining pattern. The normal adjacent colonic mucosa showed the highest score (3.9 ± 0.22), while both the primary and metastatic adenocarcinomas showed a significant decrease in BRMS1 expression compared to normal mucosa (P<0.001). While the metastases had the lowest mean score (2.5 ± 0.25), there was no statistical difference between primary and metastatic adenocarcinoma. Additionally, no difference in BRMS1 expression was noted between grade 1, 2, and 3 primary adenocarcinomas.
Conclusions: A dramatic reduction in BRMS1 expression in primary and metastatic colorectal adenocarcinomas as compared to normal adjacent mucosa suggests a critical role for the BRMS1 protein in the regulation of tumor progression and metastasis. Additionally, the lack of a significant difference in expression profile among different grades of primary tumor suggests that BRMS1 may have a role early in the tumor progression cascade. Currently, studies using survival data are underway to address the prognostic and potential therapeutic utility of BRMS1 in advanced and metastatic colorectal carcinoma.

655 Frequent Loss of Heterozygosity of a DNA Repair Gene, hOGG1, in Inflammatory Bowel Disease

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Background: Inflammatory bowel disease of the colon is an inflammatory process with a documented risk of epithelial dysplasia and development of adenocarcinoma. The hallmark of inflammatory bowel disease is persistent chronic active colitis, in which the recuited inflammatory cells can produce reactive oxygen species, generating a state of oxidative stress and frequently oxidative damage in DNA. Among various oxidative DNA lesions, 8-oxoguanine is by far the most abundant and mutagenic, if not sufficiently repaired. The gene encoding human 8-oxoguanine DNA glycosylase 1 (hOGG1), capable of excision repair of 8-oxoguanine, showed frequent gene loss or loss of heterozygosity (LOH) in a variety of human malignant neoplasms, such as head and neck squamous cell carcinoma and renal cell carcinoma. However, no study has been done to illustrate whether or not this gene will be altered in the course of inflammatory bowel disease.
Design: A total of 12 cases with established diagnosis of inflammatory bowel disease were included in the study. Twelve pairs of inflamed and adjacent normal colonic mucosa were used for microdissection of tissue. DNA samples were then obtained from dissected tissue sections. These DNA samples (24) were subjected to PCR amplification using 4 fluorescent-labeled microsatellite makers (D3S1289, D3S1297, D3S1300 and D3S1274), followed by fragment analysis using ABI PRISM 3100 Genetic Analyzer.
Results: All 12 cases are informative with at least one of 4 microsatellite makers used. Among these 12 cases, 7 (58.3%) cases showed evidence of LOH in at least one of the 4 markers used and the remaining 5 (41.7%) displayed retention of heterozygosity (RH) at the hOGG1 gene locus.
Conclusions: Loss of heterozygosity (gene loss) of the hOGG1 gene frequently occurs in inflamed colonic mucosa (58.3%) in inflammatory bowel disease. Thus, the combination of oxidative stress commonly associated with inflammatory conditions and defects in repair gene involved in oxidative DNA damage may be one important molecular mechanism by which colonic epithelium undergoes in the malignant transformation which is frequently observed in inflammatory bowel disease.

656 Ischemic Pouchitis (IP): A Histologic Evaluation and Comparison to Crohn's Disease of the Pouch (CDP) and Antibiotic-Responsive Pouchitis (ARP)

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Background: In pts with presumed ulcerative or indeterminate colitis, the long-term post-operative complications of IP, CDP, or ARP that may occur after restorative proctocolectomy with ileal pouch-anal anastomosis have different therapeutic implications. Thus, we examined the histologic features of IP to find those that may aid in its diagnosis.
Design: 10 pts with a clinical and endoscopic IP diagnosis, evidenced by efferent limb endoscopic inflammation that was sharply demarcated from the normal afferent limb at the suture line, were compared to 13 CDP and 14 ARP pts. Each pouch biopsy was examined for ischemic-type histology such as geographic epithelial atrophy, hemorrhage, and hyaline fibrosis in addition to ulceration, pyloric gland metaplasia (PGM), granulomas, and yellow-brown extracellular pigment. Mucosal injury features, including architectural distortion, mononuclear and eosinophil lamina propria (LP) cellularity, and epithelial infiltration by neutrophils (activity), were graded on a 0-3+ scale.
Results: The results are summarized in the Table. Even in pts with clinical ischemia, typical ischemic-type histologic injury was not identified. There were no significant differences between the IP and CDP groups. The IP group demonstrated significantly less activity and more frequently had associated pigment than the ARP group. None of the pigment stained with Prussian blue; none of the IP group showed PGM.

	IP	CDP	P-value	ASP	P-value
Mean age in years (range)	39.5 (29-67)	32.2 (16-48)	0.1	42.6 (19-76)	0.6
Males/Females	7/3	7/6	0.67	7/7	0.42
Mean activity score	0.9	1.0	0.9	1.6	0.007
Mean villous blunting score	1.6	1.4	0.3	2.2	0.1
Mean crypt distortion score	1.4	1.3	0.6	1.9	0.09
Mean LP mononuclear score	1.4	1.5	1.0	1.9	0.1
Mean LP eosinophil score	1.4	1.8	0.4	1.8	0.3
Granulomas	0/10 (0%)	2/13 (15%)	0.5	0/14 (0%)	1.0
PGM	0/10 (0%)	2/13 (15%)	0.2	2/14 (14%)	0.5
Ulcer	4/10 (40%)	2/13 (15%)	0.3	3/14 (21%)	0.4
Pigment	8/10 (80%)	5/13 (38%)	0.09	1/14 (7%)	0.0005

P-values are in comparision to IP
Conclusions: Aside from the extracellular pigment, specific features of IP could not be found. We are unsure of the nature of the pigment, but it may be helpful in differentiating between IP and ARP.

657 Frequent Overexpression of HMGA1 and HMGA2 in Gastroenteropancreatic Neuroendocrine Tumors and Its Relationship to *let-7* Down-Regulation

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Background: The molecular pathogenesis of gastroenteropancreatic (GEP) neuroendocrine tumors (NETs) remain to be elucidated. High mobility group A (HMGA) proteins play important roles in the regulation of transcription, differentiation, and neoplastic transformation and expressed during embryogenesis and in various benign and malignant tumors. Recent studies report that HMGA2 is negatively regulated by the *let-7* microRNAs family *in vitro.* To our knowledge, there are no reports about any miRNA expression in GEP NETs and no investigation about the relation between HMGA1 and 2 proteins expression and the *let-7* expression in GEP NETs.
Design: We performed immunohistochemistry and RT-PCR for HMGA1 and 2 expression in 55 GEP NETs from different sites and *let-7* was also analysed by semiquantitative RT-PCR. Lasermicrodissection has been performed to take tissues from tumor and non-tumor area, respectively. The data were correlated with relevant clinical information and investigated possible associations between HMGA1, or 2 expression and *let-7* expression.
Results: Overexpression of HMGA1 and 2 was frequently detected in GEP NETs compared with normal tissues. Nuclear immunostaining of HMGA1 and 2 were observed in GEP NETs (38 of 55, 69%; 40 of 55, 73%, respectively). HMGA2 expression increased from well-differentiated NET to well-differentiated NEC and poorly differentiated NEC ($P < 0.005$). In WNECs, the expression of HMGA1 and 2 was significantly higher in metastatic tumors than those without metastasis ($P < 0.05$). GEP NETs showed the highest level of HMGA1 and 2 expressions in foregut. MIB-1 labeling index correlated with HMGA1 and 2 overexpression ($R = 0.28$, $P < 0.05$; $R = 0.434$, $P < 0.001$; respectively) and progressively increased from WNETs to WNECs and PNECs ($P < 0.001$). *Let-7* expression was addressed in 6 normal organs, 30 tumor samples and 24 tumor margin non-tumor tissues. Compared with normal tissues, *let-7* down-regulation was frequent in NETs (19 of 30, 63%). Higher expression of HMGA1 and 2 was frequently observed in tumors with *let-7* significant reduction (53%, 42%, respectively). The reverse correlation could be detected between HMGA1 and *let-7* ($P < 0.05$).
Conclusions: Our findings suggested that HMGA1 and 2 overexpression and *let-7* down-regulation may contribute to pathogenesis of GEP NETs.

658 Overexpression of Notch-1 Correlates with Grade, Stage and Overall Survival in Colorectal Adenocarcinoma (CRC)

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Background: Notch signaling is believed to play a crucial role in cell differentiation, proliferation and survival. Dysregulation of Notch signaling has been reported in a wide variety of human malignancies including breast, cervical, CNS, lung, and pancreatic cancer as well as melanoma and certain leukemias. There is recent evidence that Notch signaling contributes to the natural progression of CRC. However, the prognostic significance of Notch-1 expression in CRC has not been previously investigated.
Design: Formalin-fixed, paraffin-embedded sections from 125 colorectal adenocarcinomas (CRCs) were immunostained by an automated method (Ventana Medical Systems; Tucson, AZ) using polyclonal Notch-1 antibody (sc-6014; Santa Cruz Biotechnology, Santa Cruz, CA). Cytoplasmic immunoreactivity was semiquantitatively evaluated based on both intensity and distribution and results were correlated with histologic and prognostic variables.
Results: Intense, diffuse overexpression of Notch-1 was observed in 45% (57/125) of CRC cases and correlated significantly with increasing AJCC stage (24% of stage I, 58% of stage II, 35% of stage III, and 66% of stage IV; p=0.041); histologic grade (11% of grade 1; 52% of grade 2, and 50% of grade 3; p=0.006), and overall survival (27% in those alive, 53% in those expired; p=0.011). On multivariate analysis, only pathologic stage was an independent predictor of overall survival.
Conclusions: Notch-1 overexpression is associated with tumor aggressiveness in CRC and significantly correlates with increasing tumor grade, pathologic stage and overall survival. Notch-1 overexpression may be a valuable prognostic indicator that can be used to plan therapy in CRC. Further study of Notch-1 expression in CRC management and targeted therapy development appears warranted.

659 Micropapillary Carcinoma of the Stomach: A Clinico-pathologic Study of 12 Cases

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Background: Micropapillary carcinoma (MPC) is associated with poor prognosis and extensive lymph node metastases. MPC has been reported in the breast, urinary bladder, ureter, lung, ovary, colorectum and salivary gland. However, only one case was reported in the stomach. This study aims to evaluate clinicopathologic features of MPC in the stomach.

Design: 139 gastric adenocarcinomas having features of "micropapillary" or "papillary" were retrieved from surgical pathology files among 12,867 gastrectomy and 827 endoscopic resection specimens. To exclude mucinous adenocarcinoma and papillary adenocarcinoma within lymphatic spaces, PAS and D2-40 immunohistochemical staining were performed. Under strict pathologic criteria, 12 MPCs were diagnosed; 10 from gastrectomy specimens and 2 from endoscopic resection specimens. The clinicopathologic features and immunohistochemical staining results for p53 and Ki-67 were also analyzed.

Results: We identified 12 cases of MPCs (approximate prevalence=0.08%). Nine occurred in the body and 3 in the antrum with a mean size of 5.5cm (range 0.7 to 12). Six cases showed invasion into sbumucosa and 2 each case invaded into proper muscle, subserosa and serosa. Ten cases showed differentiated type tubular or papillary adenocarcinoma in the superficial portion of the tumor. The remaining 2 cases were composed solely with poorly differentiated adenocarcinoma. The proportion of MPC within tumors varied from 5% to 70%. The endolymphatic tumor emboli were found in all cases while lymph node metastases were found in 6 cases (55%) among 11 patients who underwent lymph node dissections. Lymph node metastasis was not observed in almost all T1 carcinomas. Only tumors with more than T2 stage showed extensive lymph node metastasis. On immunohistochemistry, p53 was positive in 9 cases (75%) with strong intensity and Ki-67 proliferation index was high in most of cases (range 10 to 80%). Unexpectedly, staining intensity and percentage of staining for p53 and Ki-67 were similar in both micropapillary and ordinary adenocarcinoma areas. During follow up, two patients were died of disease and one patient showed liver metastasis even after completion of adjuvant chemotherapy.

Conclusions: MPC of the stomach is very rare, but occurs early during tumor progression and associated with more frequent endolymphatic tumor emboli. But the lymph node metastasis is closely associated with T stage rather than proportion of micropapillary patterns.

660 Multicentric Validation of a Model Based on Phenotypic Features To Predict Microsatellite Instability in Colorectal Adenocarcinomas

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Background: High microsatellite instability (MSI-H) allows the identification of a subset of colorectal adenocarcinomas (CRC) associated with good prognosis and Lynch syndrome. The objective of this work was to assess the interobserver variability of a MSI-H prediction model based on phenotypic features. This model was previously generated using a series of 204 cases.

Design: The validation series from five different hospitals included 265 primary CRC. The eight parameters that integrate our model, namely tumor location, growth, solid, mucinous and cribiform patterns, presence of Crohn-like reaction, Ki-67 and p53 immunophenotype, were evaluated in the corresponding centers and our prediction model applied to each case. The instability status was evaluated using a panel of 11 microsatellites. Simultaneously a prospective series of 148 cases was collected and identically assessed in our institution.

Results: Homogeneity assessment revealed significant differences between hospitals in the estimation of Ki-67, Crohn-like reaction, growth and cribiform patterns. Despite this observation, our model was globally able to predict MSI-H with a negative predictive value of 97.0%. The results obtained with the prospective series of 148 cases collected in our institution were equivalent, achieving a negative predictive value of 97.8%.

Conclusions: The complexity of the MSI study has triggered the development of models based on pathological features to predict instability status. A recently published model looks at pathology features associated with MSI in a series of CRC diagnosed before age 60. The model we present focus on a non-selected population of patients aiming at identifying not only possible Lynch syndrome candidates, but any CRC exhibiting an MSI-H phenotype and thus a better prognosis. The high negative predictive value achieved by our model allows the reduction of the cases to be tested for MSI to less than 10%. Furthermore, the easy evaluation of the parameters included in the model renders it a useful tool for the routine practice.

661 Microscopic Gastrointestinal Stromal Tumors (mGIST): A Series of 79 Cases

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Background: mGIST are common incidental findings in the stomach of elderly population (20-35%). Conversely, very few cases of mGIST have been reported in small intestine, large bowel and rectum. *KIT*/*PDGFRA* mutations have been detected in less than half of gastric mGIST analyzed, but whether this reflects the occurrence of other unknown initiating molecular events or it is rather the result of technical problems is unclear.

Design: 79 GIST with a diameter < 1 cm were retrieved from the files of Pathology Department of Treviso and from other 4 Italian centers. Follow-up was available for 26 patients (1 to 15 years). Cell type, cellularity, regressive changes and mitoses were evaluated. KIT immunostain was performed in all cases.In addition, 14 cases were stained for DOG1,PKCtheta,nestin,L1. *KIT* and *PDGFRA* genes status was assessed in 19 cases. DNA extraction was preceded by tumor dissection.

Results: Of 79 mGIST, 58 were located in the stomach, 16 in the small intestine, 3 in the large bowel and 2 in the rectum. Size ranged between 0.2 and 1 cm (median 0.75). The morphology was spindled in 64 cases (81%), mixed in 11 (14%) and epithelioid in 4 (5%). Cellularity was low in 39 cases (49%), moderate in 25 (31.5%) and high in 15 (19.5%). Regressive changes, as hyalinization either alone or associated to calcification, were seen in 45 cases (57%). 19 cases showed mitotic activity, with 4 cases displaying >5 mitoses (mean 0.8).Of the 21 non gastric mGIST,only 2 cases were hypocellular and hyalinized and mitotic count was significantly higher than in gastric mGIST (mean 1.3). KIT was positive in 70 cases (88.6%) and negative in 9 (all gastric). DOG1, nestin and L1 were expressed in all the 14 cases studied, whereas PKCtheta was expressed 9/14 cases. *KIT* mutation was detected in 15 of 19 cases and involved exon 11 in 13 cases and exon 9 in 2. *PDGFRA* mutation was detected in 2 cases, involving exon 18 in 1 case and exon 12 in 1 case. One of 19 cases was WT for all exons and in 1 case the results were not conclusive. Interestingly, 550-558del involving the intron 10-exon 11 boundary was over-represented (3 of 13 cases). Furthermore, one of the 2 exon 9 mutations was a point mutation (F504L), never reported before. Clinically 1 of 26 patients progressed.

Conclusions: 1. Non gastric mGIST appear as morphologically distinct from gastric ones. 2. In contrast with previous reports, *KIT/PDGFRA* mutations represent a common genetic event.

662 Clinical Significance and Follow-Up of Patients with Duodenal Biopsy Revealing Intraepithelial Lymphocytosis with Preserved Villous Architecture

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Background: Gluten sensitive enteropathy (GSE) is the most common cause of malabsorption in Europe and North America with a prevalence of 1%. Patients with GSE can develop many different clinical presentations (anemia, bloating, diarrhea, weight loss) and a spectrum of histologic findings can be seen in their duodenal biopsies. While the classic duodenal lesion consists of villous loss, intraepithelial lymphocytosis and crypt hyperplasia, patients with symptomatic GSE can have little to no histologic abnormality in the duodenum. Given this clinical and histologic variability, GSE is underrecognized. We sought to investigate the clinical significance of the histologic finding of preserved villous architecture with intraepithelial lymphocytosis in duodenal biopsies.

Design: We identified 63 patients who underwent duodenal biopsy and were noted to have preserved villous architecture and increase in intraepithelial lymphocytes (PVAIEL) on histologic examination. The histologic findings correspond to the type I lesion from the modified Marsh–Oberhuber classification of GSE. Information regarding endoscopic findings, laboratory results [GSE associated serologies: tissue transglutaminase(T), endomysial (E), and gliadin, IgG and IgA (G) antibodies], and pertinent clinical information were obtained.

Results: Of the 63 patients noted to have PVAIEL on duodenal biopsy 53 (84%) had no duodenal abnormalities on endoscopy and 23 (37%) had follow-up GSE associated serology performed (10 T&E; 6 T; 4 T,E,G; 1E; 1E&G; and 1 T&G). All follow-up serologies were negative. Ig A measurement was performed on 13 patients with only one patient having low levels. Determination of HLA-DQ type was performed in 4 individuals revealing DQ2/3, DQ7/8, DQ7, and DQ9. None of the patients were considered to have GSE with a mean of 8.6 months (0-20 months) follow-up. Review of clinical history revealed that 2 of the 64 patients had prior diagnosis of GSE and 1 of 64 had positive GSE serologies performed at an outside institution.

Conclusions: Identification of PVAIEL in duodenal biopsies in patients with a low pre-test probability for GSE may have limited clinical significance. While endoscopy with biopsy remains the gold standard for the diagnosis of GSE, appropriate GSE-associated serological tests, HLA-DQ determination, and documentation of response to a gluten-free diet are important adjunctive tests when endoscopy and biopsy results are inconclusive.

663 IL-8 Pathway in Gastric Cancer

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Background: IL-8 is a chemotactic factor for neutrophils, and has two receptors, CXCR1 and CXCR2. There have been several reports that the IL-8 pathway has a role in tumorigenesis of some cancer types. This study was conducted to determine the expression levels of IL-8 and its receptors, the relationship of IL-8 expression with inflammatory cells, and IL-8 polymorphism in gastric cancer.

Design: RNA and DNA were extracted from 70 pairs of gastric cancer and adjacent normal gastric tissues, 8 gastric cancer cell lines (AZ-521, FU-97, GTL-16, KATO-III, MKN-45, NUGC-3, OCUM-1, and SNU-1), 1 endothelial cell line (HuVEC), and a buffy coat from a healthy volunteer. Multiplex real time RT-PCR and genomic qPCR were performed to detect mRNA expression levels of IL-8, CXCR1, and CXCR2, and to identify IL-8 polymorphisms. 18S rRNA was used to normalize target mRNA. The cell proliferation assay was conducted with exogenous IL-8 and IL-8 blockers in gastric cancer cell lines.

Results: Among seventy pairs of gastric tissues, many showed high tumor vs. normal mRNA ratios of IL-8 and its two receptors; the IL-8 mRNA expression levels of tumor tissues were median 40-fold (range, 0.4~857) compared to those of adjacent normal tissues, and 50 out of 70 pairs (71%) showed over 10-fold IL-8 mRNA tumor/normal ratio. The tumor/normal ratios of IL-8 mRNA showed a close correlation with those of its two receptors, i.e, CXCR1 (r^2=0.54, P<0.001) and CXCR2 (r^2=0.47, P<0.0001), and a tendency to correlate with clinical stage. Seven out of 8 gastric cancer cell lines showed IL-8 mRNA expression. However, mRNA of CXCR1 or CXCR2 was not detectable in 8 gastric cancer cell lines and the endothelial HuVEC cell line, while mRNA of IL-8 receptors was highly expressed in a buffy coat. Neither exogenous IL-8 nor IL-8 blockers had an effect on cell proliferation of gastric cancer cell lines. Thirty nine (55.7%) out of 70 gastric tumors had AA (n=13) or AT (n=26) polymorphisms at IL-8-251, and showed a high expression level of IL-8 mRNA compared to a TT polymorphism (P=0.052).
Conclusions: Most gastric cancer tumor tissues express high levels of IL-8 and its two receptors. The IL-8-251 T>A polymorphism correlates with high expression of IL-8. Most gastric cancer cell lines showed IL-8 expression, but there is little or no expression of IL-8 receptors in those cell lines, which suggests that *in vivo* IL-8 may be working through a paracrine mechanism rather than an autocrine mechanism.

664 Utility and Limitations of MUC6 Immunohistochemistry for the Diagnosis of Sessile Serrated Adenomas

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Background: Sessile serrated adenomas(SSA) are distinguished from typical hyperplastic polyps(HP) by abnormal maturation and/or proliferation. SSA may be precursor of microsatellite unstable colorectal cancer and hence warrant surveillance similar to adenomatous polyps. However,the morphological distinction of SSA from HP can be difficult. Aberrant expression of MUC6, a gastric type mucin, has been reported in SSA but not in other types of serrated polyps. This study evaluates the specificity of MUC6 expression for SSA and explores its utility for routine diagnostic use.
Design: 72 colorectal polyps were classified as HP, SSA, traditional serrated adenoma(TSA) and tubular adenoma(TA) by 3 experienced GI pathologists using previously published criteria. A consensus diagnosis was reached in the discrepant cases by collective review. The polyps with suggestive but not unequivocal features of SSA were designated as probable SSA. Immunohistochemistry for MUC6 was performed in all cases and scored as negative,focal positive (≤5 crypts) and diffuse positive (>5 crypts).
Results: There was agreement in 58(81%) cases; a consensus diagnosis was established in the rest. There were 15 HP, 20 SSA, 10 probable SSA, 17 TSA and 10 TA. MUC6 expression was predominantly in the basal crypts and significantly more common in SSA compared to HP(p=0.02) and TSA(p<0.001). All SSA and probable SSA on the right side were MUC6+. Negative MUC6 was seen in 3 SSA and 2 probable SSA, all of which were located in the left colon. One SSA showed dysplasia; both the SSA and the dysplastic areas showed diffuse MUC6 expression. Diffuse staining was seen in 13(65%) SSA and 5(50%) probable SSA compared to 2(13%) HP and 2(12%) TSA. Of the 7 MUC6+ HP, 5(71%) were on the right, including both cases that were diffuse MUC6+.

	HP (n=15)	SSA (n=20)	Probable SSA (n=10)	TSA (n=17)	TA (n=10)
MUC6 +	7 (47%)	17 (85%)	8 (80%)	5 (29%)	1 (10%)
MUC6 diffuse +	2 (13%)	13 (65%)	5 (50%)	2 (12%)	1 (10%)

Conclusions: MUC6 expression has high sensitivity (>80%) for the diagnosis of SSA, and is positive in all right-sided cases. The staining is diffuse in majority of SSAs. Less often, MUC6 expression can be seen in HP and TSA, with focal staining in majority of cases. Diffuse staining can occur in approximately 10% of HP and TSA. Although not entirely specific, MUC6 immunohistochemistry can be useful for the diagnosis of SSA, especially if diffuse expression is obtained.

665 High Frequency of E-Cadherin Methylation and Loss of Expression in Colorectal Signet Ring Cell Carcinoma Compared to Mucinous and Nonmucinous Adenocarcinoma

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Background: Signet ring cell carcinoma(SRC) is a rare subtype of colorectal cancer. Its aggressive behavior may be related to discohesive tumor cells facilitating spread of the disease. Loss of E-cadherin, an important adhesion molecule, can enable metastasis by disruption of intercellular contact.The E-cadherin status in SRC and its comparison with mucinous(MC) and nonmucinous adenocarcinoma(AC) has not been studied.
Design: E-cadherin status was determined by immunohistochemistry(IHC) and methylation in 48 colorectal carcinomas(17 SRC,17 MC,14 AC). The staining was recorded as positive (≥20%) or negative (<20% tumor cells). In addition, the positive cases were divided into diffuse (>75%) and decreased expression (20-75% tumor cells) for SRC. Methylation status was determined by methylation-specific polymerase chain reaction. The relationship of E-cadherin status in SRC with clinicopathologic features as well as with previously determined molecular features like microsatellite instability (MSI), CpG island methylator phenotype (CIMP), BRAF mutations and KRAS mutations was examined.
Results: Loss of E-cadherin expression by IHC and E-cadherin methylation was seen more often in SRC compared to MC and AC(see table). SRC with E-cadherin methylation showed absent(60%) or decreased(30%) expression. Two cases of SRC without methylation also showed loss of E-cadherin. Distant metastasis was seen more often in E-cadherin negative SRC(29% vs 0%, p=0.02). There was no correlation between E-cadherin loss or methylation and age, gender, site or molecular features like MSI, CIMP, BRAF mutations and KRAS mutations.

	SRC	MC	AC	p value
E-cadherin expression	76	18	21	0.002, 0.008
E-cadherin methylation	79	13	47	<0.001, 0.05

Figures reflect percentages;p values are SRC vs MC, SRC vs AC
Conclusions: Methylation and loss of E-cadherin expression is observed in a vast majority of SRC compared to a minority of MC and AC. Most cases with methylation show loss or decreased E-cadherin expression. E-cadherin loss was also observed in the absence of methylation, suggesting alternative mechanisms of E-cadherin down regulation. Metastatic disease was observed exclusively in E-cadherin negative SRC. Loss of E-cadherin may breach cell to cell adhesions resulting in the discohesive appearance typical of SRC and may play a role in facilitating distant metastasis.

666 PCSK1 Expression Correlates with Improved Prognosis in Colorectal Carcinoma

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Background: There is a need for reliable prognostic biomarkers in colorectal carcinoma (CRC), especially for low stage disease. In this study, we used CRC oligonucleotide microarray expression profile data to identify transcripts that predict disease progression. To validate the biological significance of identified transcripts we investigated the protein expression on tissue microarrays (TMAs).
Design: The expression profile of 147 primary CRC was obtained using HG-U133A microarrays. To select transcripts that correlate with prognosis, the stage I to III CRC were divided in good and dismal prognostic groups (no recurrence at 5 yr -53 cases vs recurrence or death of disease within 5 yr from diagnosis -16 cases, respectively). Transcripts demonstrating >5 fold difference and t-test p< 0.01 between the two groups were selected. Protein expression was investigated by immunohistochemistry on 3 TMAs containing a total of 225 cases of CRC. Cases demonstrating a 1+ to 3+ staining intensity in more than 5% of tumor cells in at least one core were regarded as positive. Kaplan-Meier curves were computed to assess correlation with recurrence and survival between the positive and negative groups.
Results: PCSK1 transcript was overexpressed by 12.4 folds in indolent versus aggressive CRCs (p=0.006). PCSK1 (proprotein convertase type 1) is involved in the release of protein hormones and neuropeptides from their precursors and is expressed in endocrine organs and brain. Out of 99 cases of stage I to III primary CRC, 39 tumors expressed PCSK1 protein. The PCSK1-positive cases showed an improved overall survival. For stage I to III CRCs, the 5- and 10-year survival rates in PCSK1-positive versus negative cases were 94.5% and 83.3% versus 86.3% and 59.4% respectively (p=0.05). The 5- and 10-year disease-free rates in PCSK1-positive versus negative cases were 82.9% and 82.9% versus 73.9% and 66.9% respectively however, statistical significance was not reached (p=0.1). When only stage I and II CRC tumors were analyzed, the 10-year survival rate was 100% in the PCSK1-positive group compared with 81% in the negative one.
Conclusions: In our study, expression of PCSK1 protein stratified low stage CRC into 2 groups with different clinical outcomes. This is the first report suggesting an association between PCSK1 and neoplastic progression in CRC. Further validation studies are necessary to confirm the clinical relevance of PCSK1 expression and to elucidate its biological significance in colonic carcinogenesis.

667 The Type of Host Inflammatory Immune Response Predicts Survival in EBV-Associated Gastric Carcinomas: A Multivariate Analyses of 109 Cases

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Background: Epstein-Barr (EB) virus is detected in about 10% of gastric carcinomas (GCs) and the association between EBV infection and GC is well established. Most EBV-associated GCs are lymphoepithelioma-like carcinoma (LELC), but 6.9% to 16% are ordinary adenocarcinomas. EBV-associated GCs tend to be proximally located, of the diffuse subtype, and have lower frequency of lymph node metastasis. The aim of this study was to explore the relationship between host inflammatory immune response and prognosis in EBV-associated GCs.
Design: 109 EBV-associated GCs were evaluated by EBV in-situ hybridization. The pathologic features, TNM stage, metastatic lymph node ratio (LNR; total positive LNs / total LNs examined), disease-free survival, and overall survival were analyzed. Overall and disease-free survivals were calculated using the Kaplan-Meier method.
Results: EBV-associated GCs revealed three pathologic subgroups; typical LELC with prominent lymphoid stroma (n=54), carcinoma with lymphoid follicles simulating Crohn-like reaction (CLR) (n=42), and ordinary adenocarcinoma (n=13). LELCs were associated with less frequent lymphatic invasion, more frequent perineural invasion, lower T and N stages and LNR compared to GCs with CLR or ordinary adenocarcinomas (p<0.05). Upon univariate analyses, histologic subtype (LELC), lower LNR and T and N stages were all closely associated with longer disease-free survival and overall survival (p<0.000). Upon multivariate analyses, histologic subtype (p=0.001, hazard ratio 4.075) and T stage (p=0.001, hazard ratio 3.126) were independent prognostic factors.
Conclusions: EBV-associated GCs have three distinct histologic subtypes and these subtypes were closely associated with prognosis. Our results indicate that the prognosis of EBV-associated GCs may be related to the type of host immune response to EBV.

668 Mitosin Expression Is a Biomarker of Cancer Progression in Barrett's Esophagus

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Background: Molecular markers of cancer development in Barrett's esophagus (BE) include 17p (p53) LOH, tetraploidy and aneuploidy. We have previously identified a more than 5-fold increase in the expression of mitosin (CENPF) by mRNA microarray in p53 -/- tetraploid (G2/4N) epithelial cell strains established from BE biopsies (Barrett et al. Cancer Res 15:63 (14):4211-7). Mitosin is a nuclear matrix protein that plays a major role in chromosome segregation during mitosis and is, thus, important for cell cycle control. The aim of this study was to evaluate mitosin as a biomarker of cancer progression in BE by comparing its expression in patients who progressed to dysplasia / cancer, versus those who did not, over the course of long-term endoscopic surveillance.

Design: Routinely processed non-dysplastic index biopsies of 46 BE patients (M/F ratio: 35/11, mean age: 56 years), consisting of 19 patients who progressed to dysplasia (N=6) or cancer (N=13) over the course of long-term surveillance (mean follow-up = 58.3 months; range 13 - 160 months) and 27 patients who did not develop dysplasia or cancer upon follow-up (mean follow-up = 123.9 months; range 35 - 226 months) were immunohistochemically stained for mitosin and evaluated in a blinded fashion for the mean number of positive cells per crypt in each biopsy specimen. Comparisons were made between progressors and non-progressors, and also between the subgroups of dysplasia and cancer progressors.

Results: BE progressors showed significantly increased mitosin expression in index biopsies (mean # positive cells/crypt =17.5 +/- 5.5) versus non-progressors (14.2 +/- 5.9, p=0.04). Of the neoplasia progressors, there was no significant difference in expression between patients who developed cancer compared to those who only progressed to dysplasia (low or high-grade). When only patients who progressed to cancer (n=13) were compared to non-progressors, the mitosin grade remained significantly higher in the former (17.6 +/- 5.6) compared to the latter (14.2+/-5.9, p=0.05).

Conclusions: Mitosin is involved in the pathogenesis of cancer in BE and overexpression may represent a potentially valuable biomarker in patients with this disorder. Further prospective studies on larger cohorts of BE patients should be performed in order to further evaluate this finding.

669 Colonic Crohn's Disease: A Clinical/Pathologic and Outcome Study of 120 Patients

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Background: We have noted, anecdotally, that the clinical and pathologic manifestations of Crohn's disease (CD) of the colon are different in patients either with or without ileal involvement, but this type of analysis has never been performed. Thus, the aim of this study was to evaluate the clinical, pathologic and outcome features of the colon in patients with isolated colonic CD (ISCD) versus those with ileocolonic CD (ICCD), at initial presentation.

Design: Colonic resection specimens from 79 patients with ISCD and 41 patients evaluated during the same time period with ICCD, at initial presentation, were recruited from the files of 3 major university hospitals. All patients were evaluated for a wide variety of clinical and pathologic features (the latter also separated into major and minor CD features) and also for outcome (favorable or unfavorable) after surgery, in a blinded fashion without knowledge of the patient group. Major CD pathologic features included granulomas, transmural lymphoid aggregates, fissuring ulceration (FU), sinus tract formation, fistulas (fist), anal involvement, and segmental disease.

Results: Clinically, patients with ISCD were significantly older (37 vs. 26 years, p=0.006), but did not differ with regard to gender or race. Grossly, the colon from ISCD patients revealed a significantly lower percentage of stricture/stenosis (p=0.003), adhesions (p<0.001), fist formation (p=0.01), a higher proportion of cases with worse disease in the distal vs. proximal colon (p<0.001), a higher rate of left sided colitis (p<0.001), overall lower severity of disease (p=0.001), a lower prevalence rate of FU (p<0.001), and a nearly significant increased rate of anal involvement (p=0.09) compared to the colon of patients with ICCD. In addition, ISCD patients showed significant fewer major CD features compared to ICCD. No differences in colonic dysplasia were noted. Upon follow-up, no significant differences were noted between the two groups with regard to an unfavorable outcome.

Conclusions: The colon in patients with ISCD at initial presentation show less frequent classic CD features and, thus, resemble ulcerative colitis more frequently than the colon of patients with ICCD. However, outcome after surgery is similar and, thus, ISCD and ICCD should be managed similarly.

670 Helicobacter Pylori Detection in Gastric and Oral Mucosa

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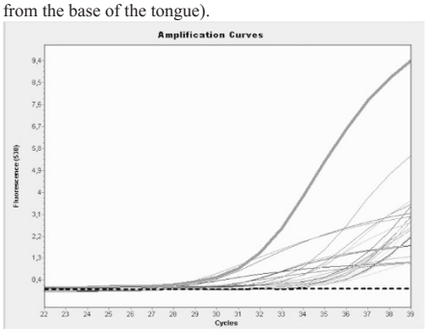
Background: Helicobacter pylori (H. pylori) infection has been related to various gastroduodenal pathologies like gastritis, peptic ulcer and carcinoma. Some authors have reported that the oral cavity could be a reservoir for the bacteria in patients with gastric H. pylori and thus, its detection in the oral cavity could represent a non-invasive method to detect its presence. Conventional methods have failed in the detection of H. pylori in the oral cavity, maybe because of its low counts. The objective of this study was to detect H. pylori in the oral cavity of patients with positive cultures for H. pylori of gastric biopsies.

Design: Fifty-four patients with medical indication of digestive endoscopy from the Gastroenterology Unit of the Regional Hospital of Concepción, Chile, were studied. Gastric samples were obtained from each patient from antrum and corpus through endoscopy biopsies and studied by culture. Positive samples were genetically verified by conventional PCR. Oral samples from all patients with positive gastric cultures for H. pylori (n=21) were obtained from dental plaque and saliva swabs from the floor of the mouth and the base of the tongue. All oral samples were studied by culture, conventional PCR and Real Time PCR.

Results: All cultures from oral samples were negative (0/21) for H. pylori. Only one sample of dental plaque was positive with conventional PCR (1/21), while all samples of saliva were negative. However, samples from all patients were positive with Real Time PCR (20/21 dental plaque, 21/21 saliva from the floor of the mouth, 20/21 saliva from the base of the tongue).



Quantification showed that there is 1x103 less bacteria in the mouth than in gastric samples.

Conclusions: The results suggest that there is a correlation between the presence of H. pylori in gastric mucosa and the oral cavity. Also, that Real Time PCR is the best technique to detect low numbers of bacteria in the oral cavity.

671 The Prognostic Significance of Human Papillomavirus (HPV) in Invasive and In Situ Squamous Cell Carcinoma (SCC) of the Anus

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Background: HPV, particularly HPV 16, plays a strong role in SCC of the anus. Current prognostic factors for treatment and survival do not include HPV status. However, high risk HPV has been associated with improved outcomes and response to treatment within the context of oropharyngeal SCC. We studied the impact of high risk HPV status on disease progression and prognosis in anal SCC.

Design: A computer search of a large teaching hospital identified 59 cases over 23 years (1984-2007) including invasive (n=31) and in situ lesions (n=28). Patients lacking available pathologic follow-up were excluded (n=14) yielding a total of 22 invasive and 23 in situ cases (n = 45). Chart review was conducted to determine age, sex, HIV status, and stage at initial presentation. Tissue sections from 45 cases were analyzed by p16 immunohistochemistry. Cases showing p16 expression were subjected to HPV 16-specific fluorescence in situ hybridization (FISH). HPV status was then correlated with the aforementioned patient variables.

Results: The average patient age was 47 years (25 – 86 years) with a slight male predominance (n=26). HIV status was known in 19 patients, 12 HIV positive. Among invasive lesions 81% (n=18) showed p16 expression, with HPV 16 detected in 41% of cases. These all presented de novo as invasive SCC. Seventy percent (n=16) of in situ lesions showed p16 upregulation with detection of HPV 16 in 22% of cases. The rate of HPV 16 prevalence did not differ significantly between in situ versus invasive SCC (p=0.14). Four in situ lesions progressed to invasive SCC. Expression of p16 was detected in 1/4 progressive cases, while 3 showed no p16 upregulation. HPV 16 was absent in all 4 cases. Among those maintaining an in situ lesion 79% showed p16 expression, with only 26% having HPV16 detected. Finally, within invasive SCC, p16 upregulation and the presence of HPV 16 did not statistically correlate with disease prognosis as defined by stage. Eighty-six percent of persons with stage ≤ 2 disease, and 75% with stage > 2 disease were shown to have p16 upregulation. HPV-16 was detected in 43% of persons with stage ≤ 2 disease, and 38% with stage > 2 disease. No significant correlation was seen with HIV status.

Conclusions: HPV 16 is the most commonly associated HPV subtype with anal SCC. While HPV is a known etiologic factor in carcinogenesis, it is not a prognostic factor for disease as defined by stage in this study, nor does it influence in situ disease progression. Location, tumor size, and patient age remain the most important prognostic factors.

672 Proposal for a More Applicable and Clinically Relevant Staging Evaluation of Ampullary Carcinomas

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Background: The staging of ampullary (AMP) carcinoma (CA) is challenging due to the anatomic complexity of this region and rarity of these tumors.

Design: 98 resected invasive AMP-CAs were evaluated for T-staging parameters. In addition to depth of invasion into different structures, size of invasion (largest diameter of *non-mucosal* component) was also analyzed.

Results: An attempt was made to classify the cases by the current TNM; however, it was readily evident, especially from the 30 cases grossed using the open-ampulla approach, that this system is inapplicable and irreproducible due to problematic definition, and presumed significance of "duodenal wall", "invasion to pancreas" and "extrapancreatic soft tissue". Thus, a modified staging approach was devised according to 3 dimensional

(3-D) spread of the tumor, possible biologic significance of extension to the structures readily associated with the ampulla, and practicality and reproducibility. The ampulla was regarded as a tube in continuum with the duodenum and the following levels of tumor involvement were assigned: **T1**-submucosa (of any component); **T2**-muscles (regardless of whether it is Oddi, duodenal or CBD); **T3**-crossing the muscles into adjacent tissues; i.e duodenal subserosa (T3s) or ≤0.5 cm into the pancreas (T3p). 0.5 was chosen because it was noted that cases with subserosal involvement also had a peri-muscular spread of up to 0.5 cm. **T4**-serosal surface, or invasion into pancreas >0.5 cm. This proposed staging had significant prognostic value: Median overall survival (OS) for **T1-T4** were 122, 65, 24, 25 mos, respectively (p=0.026). OS of **T1/2** was markedly better than **T3/4** (122 *vs* 24 mos; p=0.004). Separately, size of invasive CA was found to be highly predictive of clinical outcome, as invasion ≤1.0 cm was associated with OS of 122 mos, whereas >1.0 cm was 26 mos.(p=0.004).
Conclusions: The proposed staging system incorporates various facets of the 3-D spread of AMP-CA not properly captured in the current TNM. It is practical and reproducible, and it has strong *prognostic value.* Size of invasive CA provides additional important survival information and should be included in pathologic evaluation of AMP-CA.

673 Variables Impacting Lymph Node Assessment in Colorectal Resection Specimens Removed for Adenocarcinoma

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Background: Recent data suggest that higher number of nodes evaluated in colon cancer colectomy specimens is associated with better survival, regardless of pathologic findings. Recommendations from the American College of Surgeons, the American Society of Clinical Oncology and others mandate the harvesting of at least 12 nodes in colectomy specimens for adequate assessment. Number of nodes harvested is not only being used as a benchmark for both surgeons and pathologists but is also proposed to be of significance for payment strategies to surgeons and institutions by insurance companies. Recent published studies contradict this concept and argue that the above association is complex and is uncontrolled for a variety of confounding variables. The objective of this study is to evaluate the impact of several key confounding factors on our ability to harvest at least 12 nodes per colectomy specimen.
Design: We reviewed 306 colon cancer colectomy specimens from 2 academic medical centers, including 177 from Kansas University Medical Center (KUMC) and 129 from The Kansas City Veterans Affairs Medical Center (VAMC) from 2003 to 2007. Factors evaluated included tumor size, grade, stage, site, number of positive nodes and length of colectomy segment removed. In addition we compared the number of nodes removed in the 2 sites and whether individual surgeons had an impact on number of harvested nodes.
Results: The likelihood of harvesting ≥12 nodes is correlated with larger tumor size, higher grade and stage and specimens longer than 21 cm. More nodes were harvested from the right colon (mean=13 nodes), followed by descending (12 nodes), transverse and rectosigmoid (10 nodes, each). Surgery date didn't have an impact on number of harvested nodes. Number of positive nodes correlated with tumor grade, but not with tumor site, size, linear length of specimen or 12 nodes or more harvested. More cases from the VAMC were likely to harvest at least 12 nodes compared to KUMC. That was not related to tumor size, grade, stage, tumor site or length of segment removed. It was noted however, that 2 of 10 surgeons at the VAMC performed 76% of cases. There were 21 surgeons at KUMC; none performed more than 12% of the cases.
Conclusions: Number of harvested nodes is primarily influenced by prognostically significant parameters mainly related to tumor biology. The potential impact of surgeon's experience and the type of surgery performed needs further evaluation.

674 *UEV1A* Is a Candidate Proto-Oncogene That Participates in NF-κB Activation

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Background: Activation of NF-KB signaling pathway is considered to be pro-survival and anti-apoptotic; hence, NF-KB activation could provide a hallmark phenotype for cancer. Recent observations suggest that TRAF (E3) and Ubc13-Uev1A (E2)-mediated Lys63-linked polyubiquitination plays a central role in the NF-KB signaling pathway. We hypothesize that Uev1A functions as a regulatory subunit of the Ubc13-Uev1A and TRAF-mediated NF-KB pathway and that the abnormal expression of *UEV1A* plays a critical role in tumorigenesis. Also, a universal overexpression of *UEV1A* mRNA was previously described in all tumor cell lines examined.
Design: Stable HepG2 transfectants expressing *UEV1A-myc* at different levels were established and the suppression of *UEV1* was achieved by RNAi. The *UEV1* transcript level was evaluated by RT-PCR with *UEV1A*-specific primers in 7 fresh samples of colon adenocarcinomas. We have raised mAbs specific for the N-terminal domain of Uev1A and used it for immunohistochemical (IHC) detection of Uev1A protein expression in formalin-fixed, paraffin-embedded samples of 58 patients (20 gastric and 38 colorectal adenocarcinomas with matched samples of normal mucosa and matched metastatic tumors (191 samples in toto). NF-KB activation was determined by IHC detecting nuclear translocation of its p65 subunit.
Results: 2.5-fold *UEV1A* expression resulted in an up to tenfold increase in NF-KB activity and the activation of its target genes, as assayed by Bcl-2 western blot. RNAi suppression of Uev1 reverted the NF-KB activity to below the basal level. RT-PCR showed elevated *UEV1A* mRNA in 1/4 primary colon cancers and 2/3 metastatic tumors. Uev1A protein expression was not detected by IHC in histologically normal mucosa, but it was expressed in 6/20 primary gastric and 21/46 primary and 44/56 metastatic colorectal tumors (p<0.0001, Chi-Square). There was almost perfect correlation between NF-KB activation and Uev1A (p<0.0001). Uev1A was not detected in 50% of tumors showing NF-KB activation, suggesting that in these tumors NF-KB is activated through mechanisms other than Uev1A upregulation.
Conclusions: *UEV1A* is a candidate proto-oncogene that participates in NF-KB activation and it may play a critical role in about 1/2 of gastric and colorectal tumors showing NF-KB activation. It also appears to play a positive role in tumor progression since it is more often observed in metastatic than in primary tumors.

675 MiRNA-21, a Potential Prognostic Marker for Human Gastric Cancer

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Background: MicroRNAs (miRNAs) are small regulatory RNA molecules. Recently, they have been identified in the progression of various cancers. Differential expressions of miRNA-21 (miR-21) and miRNA-29 (miR-29) have been reported and confirmed in lung, colon, and breast cancers. However, little is known regarding the expression patterns and role of miRNAs in gastric cancer.
Design: Human gastric cancer samples (n=54) and available paired normal gastric mucosa were evaluated for the expression of miR-21 and miR-29. Total RNA was extracted from microdissection tissue using a modified TRIZOL procedure. RNA (800 ng) was converted to cDNA by priming with a mixture of looped primers to miR-21 and miR-29 (Mega Plex kit, Applied Biosystems) using reverse transcription conditions. Real-time PCR was performed under standard conditions. The optimal internal control was determined by comparing the mean 2-CT of the tumor and normal groups. The relative expression of each miRNA was calculated using the equation 2-ΔCT, where ΔCT = (CTmiRNA – CTinternal control). All qRT-PCRs were performed in duplicate, and the data are presented as means ± standard errors of the means (SEM). Differential expression of miRNAs was analyzed and correlated with clinicopathological parameters, including age, gender, tumor grade, lymph node involvement, and survival data.
Results: The mean expression of miR-21 in tumor and normal samples were 27.09 and 16.26 (SEM: 22.83 vs. 8.71), respectively. The differential expression of miR-21 in tumor and normal tissue was statistically significant (p=0.0032). miR-21 was overexpressed in 32 of the 54 gastric cancer patients (59.26%). These 32 patients with elevated expression of miR-21 displayed a poorer prognosis (p<0.05) compared to those without miR-21 overexpression. No significant correlation was observed between miR-21 expression and other clinicopathological variables. The mean expression of miR-29 in tumor and normal samples were 0.1852 and 0.2035 (SEM: 0.1831 vs. 0.2013), respectively. There was no differential expression of miR-29 in tumor and normal gastric tissue (p=0.307).
Conclusions: Our study clearly demonstrated that miR-21 was significantly up-regulated in approximately 60% of gastric cancers. The unfavorable clinical outcomes observed in those with miR-21 overexpression suggest that miR-21 is a potential valuable prognostic marker for clinical assessment and management of gastric cancer.

676 Prognosis of Gastric Cancer: A Genome-Wide Study Using 244K Array Comparative Genomic Hybridization (aCGH) and Verification by Fluorescent In-Situ Hybridization (FISH)

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Background: Accumulated evidence suggests that multiple genetic alterations are involved in the complex carcinogenetic process of solid tumors such as gastric cancer (GC). Although a number of genetic changes has been reported in GC, including amplification of *CCNE, CMET, FGFR2* and *KSAM*, mutation of *E-cadherin, KRAS* and *Tp53* genes, and loss of heterozygosity on 5q, 17p, and 18q, the molecular events leading to GC and its progression remain largely unknown.
Design: Oligonucleotide array comparative genomic hybridization (aCGH) was performed on 40 microdissected GC samples using a high-density (244K) aCGH system (Agilent Technologies). Agilent's Feature Extraction 9531 and aCGH Analytics 3440 software programs were used to map the oligo's signal strength onto a build of the human genome to visualize the gain or loss (based on the reference used in the other channel on the same slide) of DNA. For each aCGH probe, each sample was classified as having normal, gained, or lost DNA copy number based on log 2 ratio thresholds of ±0.15. An independent set of tissue arrayed samples (n=63) was further validated by FISH after one focus (19q13.3) was found to have association with patients' survival. The mean patients' survival follow-up time was 58 months.
Results: aCGH identified 1271 genes with DNA copy loss and 1449 genes with DNA copy gain in gastric cancer. Among these identified genes, 1 deleted and 198 amplified genes were observed to have significant association with patients' survival. Forty-eight of these genes were specifically located on chromosome 19q13.3, including *CRX, DACT3, DKKL1, EHD2, EMP3, HIF3A, HRC, IGFL2, IGFL3, KPTN, LIG1, PNKP,* and *PTOV1*. Compared with all other patients, those (n=14) with gene amplification on 19q13.3 had a significantly poorer prognosis (p<0.01). Furthermore, using 19q13.3 probe (Vysis, Abbott, IL) by FISH method, amplification of 19q13.3 was identified in 18 cases with unfavorable clinical outcome.
Conclusions: This genome-wide study identified a panel of critical genes associated with progression of gastric cancer. Amplification of the genes on chromosome 19q13.3, a possible signature event in gastric carcinogenesis, represents a potentially useful prognostic biomarker for this aggressive malignancy. Further functional studies are needed to confirm the potential value of these genes in the management of gastric cancer.

677 Prognostic Significance of Tumor Infiltrating Lymphocytes in Colorectal Carcinoma Occurring in Sporadic and Familial Settings

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Background: Quantification of intraepithelial tumor infiltrating lymphocytes (TILs) on hematoxylin and eosin sections has been shown to be useful in predicting microsatellite instability (MSI) in sporadic colorectal cancer (CRC) and in hereditary nonpolyposis colorectal cancer (HNPCC). We examined the impact of the TIL count on survival, when MSI status and family history are taken into consideration.

Design: The study included 206 CRC cases from 204 patients. All cases were tested for MSI by PCR and classified as MSI-H if at least one third of the markers analyzed were abnormal. Tumors from patients who had a family history that fulfilled Amsterdam criteria or who had three or more CRC among first or second degree relatives were classified as HNPCC/HNPCC-like. The number of lymphocytes in tumor cells in 10 random high power fields (HPF) was recorded. Outcome data were obtained from the surgical database. Disease free survival (DFS) was estimated using the Kaplan-Meier method and Cox regression model was developed to determine the set of factors that independently predicted survival.

Results: The study population consisted of 101 males and 103 females (median age 62 yrs, range 23-84 yrs). Eighty cases (39%) were classified as HNPCC/HNPCC-like and 71 (34.5%) as MSI-H. The TIL count was significantly higher in MSI-H cases compared to cases with no or low MSI (57.8 vs. 9, $p<0.01$) and in HNPCC/HNPCC-like tumors compared to sporadic ones (42.3 vs. 15.4, $p<0.01$). A higher TIL count was also associated with younger age and lower tumor stage. In univariate analysis, low stage, family history and >5 TILs/10 HPF were associated with a significantly better DFS ($p<0.01$). The impact of MSI status on survival did not reach statistical significance ($p=0.06$). A high TIL count was associated with better DFS among both sporadic and familial CRC with HNPCC/HNPCC-like tumors showing >5 TILs/10 HPF having the best 5-yr DFS (95.7%). Multivariate analysis found high stage, family history and >5 TILs/10 HPF to be independent predictors of survival (hazard ratios 10.1, 0.43 and 0.4).

Conclusions: Quantification of intraepithelial TILs on hematoxylin and eosin sections provides significant prognostic information in sporadic and familial CRC independent of stage. The TIL count in our study had a greater impact on survival than MSI status and in combination with a positive family history identified a group of patients with a particularly favorable prognosis.

678 Etiology and Clinical Implications of Collagenous Sprue: A Single Institution Experience

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Background: Collagenous sprue (CS) is considered one of the causes of refractory sprue. The etiology of CS is controversial and this entity has been associated with poor clinical outcomes. We thus retrospectively analyzed cases of CS diagnosed at our institution.

Design: Patients were identified through a search of our departmental archives between 1999 and 2008. Small bowel biopsies were assessed for histologic variables including subepithelial collagen and degree of villous atrophy. The thickness of collagen deposition was measured on trichrome stained sections and classified as mild (10-14 μm), moderate (15-20 μm) or marked (>20 μm). Stains for CD3 and CD8 and PCR for T cell receptor gene rearrangement were performed in all cases. Flow cytometry analysis was performed in a subset. Clinical data including serologies and response to gluten free diet (GFD) and immunomodulator therapy (IMRx) were obtained from the treating physicians.

Results: Twenty one patients with CS were identified (18F, 3 M, age range 25-80 yrs, median 63 yrs). Twenty patients (95%) had positive serologies for celiac disease. One patient was diagnosed with autoimmune enteropathy. Ten patients (48%) had an atypical (non-diarrhea predominant) presentation. Small bowel biopsies from 19 patients were available for assessment of collagen thickness, which varied from mild (n=9) to moderate (n=6) to marked (n=4). The degree of villous atrophy was total (n=12) or subtotal (n=7). An increase in CD3+CD8- intraepithelial lymphocytes was observed in 42% (8/19) of the biopsies, and flow cytometry, where performed, showed an expansion of γδ T cells. PCR analysis showed minor clones in 4/19 (21%) cases. The degree of subepithelial fibrosis did not correlate with the presence of clonal T cell expansions. Follow-up biopsies, available in 10 patients showed a reduction in subepithelial collagen in 6 patients, while persistent T cell clones were seen in 2 patients. Clinically, 6/21 (29%) patients responded to GFD and all patients (n=8) receiving IMRx showed a response. None of the 4 patients with marked fibrosis responded to GFD. There was no progression to lymphoma and all patients are currently alive (4.1 yrs mean follow-up time; range 0.3-9.5 yrs).

Conclusions: The vast majority of patients in our series of CS had celiac disease. Most cases had total villous atrophy with polyclonal T cell infiltrates. A response to GFD was seen, although in a minority of patients. Even though most patients required IMRx for symptom control, CS was not associated with a dismal prognosis as reported previously.

679 Cadherin Expression in Gastrointestinal Tract Endometriosis: Possible Role in Deep Tissue Invasion and Development of Malignancy

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Background: Cadherins are cell surface proteins crucial for cell adhesion and tissue integrity. Studies of peritoneal endometriosis have shown decreased E-cadherin and β-catenin expression with preserved N-cadherin expression. We recently encountered a case of carcinoma arising in colonic endometriosis that showed total loss of N-cadherin, but preserved E-cadherin and β-catenin expression in the tumor. The mechanism of deep tissue invasion in gastrointestinal (GI) endometriosis is unknown and may be related to the expression of these cell surface proteins. The goal of this study was to evaluate the expression of E-cadherin, β-catenin and N-cadherin in GI endometriosis.

Design: Cases of GI endometriosis identified from our pathology database were included in the study. The histopathology was reviewed and the anatomic site and extent of involvement was recorded for each case. Immunohistochemistry was performed using antibodies against N-cadherin, E-cadherin, and β-catenin on 4-μ formalin-fixed paraffin-embedded representative tissue sections from each case. Cases of normal endometrium (n=5) and adenomyosis (n=6) were also included in the study for comparison. The intensity of staining for each marker was scored subjectively on a scale of 0 to 3 and the number of glands staining was scored from 0 to 4 (0 = none, 1 = 1 to 24%, 2 = 25 to 49%, 3 = 50 to 74%, 4 = 75 to 100%). Appropriate positive and negative controls were used.

Results: Twenty-one cases of GI endometriosis were included in the study (colon=11, appendix=8, small bowel=2). The endometriosis was seen infiltrating up to mucosa (n = 1), submucosa (n = 7), muscularis propria (n = 10) and only serosa (n = 3). Significantly decreased N-cadherin expression (3=0, 10=1+) was found in 13 (61.9%) cases of GI endometriosis compared to controls. Two of 3 cases involving only the serosa showed strong diffuse N-cadherin staining as has been previously reported in peritoneal endometriosis, while distinct and marked loss of N-cadherin was seen in the more deeply invasive lesions that extended into muscularis propria, submucosa or mucosa. Moderate to strong membranous staining for β-catenin expression was diffusely present in all cases. Variable intensity of E-cadherin expression was also seen diffusely in all cases.

Conclusions: These results strongly suggest that loss of N-cadherin in GI endometriosis may play an important role in the mechanism that underlies deep tissue invasion, and possibly also in the development of malignancy.

680 Follow-Up of Pancreatic Fine Needle Aspirates Diagnosed as "Atypical": A Retrospective 15-Year Review of 127 Cases

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Background: Since a false-positive pancreatic FNA can lead to unnecessary radical surgery, the "atypical" diagnostic category may be employed whenever the cytopathologist lacks complete confidence in his or her malignant diagnosis. In this study, encompassing 15 years of practice, the outcome of fine needle aspirates of the pancreas diagnosed as "atypical" were retrospectively reviewed.

Design: A computerized search of the cytology laboratory information system from July 22, 1993 through July 21, 2008 was performed and all pancreatic FNA cases diagnosed as "atypical" were identified. All correlating cytology and surgical pathology reports from these cases were reviewed as were selective cytology and surgical pathology slides.

Results: During this 15-year period, indeterminant cytologic diagnoses of "atypical" cells were rendered in 127 pancreatic FNA cases for which confirmatory follow-up material was available. There were 74 males and 53 females ranging in age from 34 to 86 (mean age: 63). In 54 cases (43%), the diagnosis of malignancy was confirmed by follow-up surgical pathology (Whipple resection-31 cases, surgical biopsy-3 cases) or cytology (repeat FNA-13 cases, brush cytology-3 cases). In 25 cases (20%), a suspected diagnosis of malignancy was confirmed by the documentation of metastatic disease by surgical pathology (23 cases) or FNA (3 cases) at the metastatic sites. In total, 79 of 127 "atypical" pancreatic FNA cases were confirmed as adenocarcinoma (62%). In 17 cases (13%), a histologic diagnosis of intraductal papillary mucinous neoplasia neoplasm (IPMN) was rendered. Other malignancies noted on follow-up included 4 cases of pancreatic endocrine neoplasia, 3 cases of metastatic carcinoma and 1 case of gastric lymphoma. A total of 22 benign cases were present among the 127 "atypical" FNA cases. These included 16 cases of chronic pancreatitis, 2 cases of serous cystadenoma and 4 other benign lesions.

Conclusions: The majority of pancreatic FNA cases diagnosed as "atypical" were proven to be adenocarcinomas (62%), other malignancies (6%) or IPMNs (13%). The high yield of malignancy following a pancreatic FNA diagnosis of "atypical" cells reflects the natural diagnostic conservatism of most cytopathologists. Because chronic pancreatitis and other benign lesions were included among the group of cases diagnosed as "atypical" by pancreatic FNA, abandonment of this category is not warranted. Careful re-examination of "atypical" pancreatic FNAs that prove to be malignant could enhance the cytopathologists' diagnostic skills.

681 ProEx C Immunostain as a Marker for Dysplasia in Barrett's Esophagus

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Background: ProEx C is an immunohistochemical stain containing monoclonal antibodies targeting the topoisomerase II-alpha and minichromosome maintenance protein 2, which are overexpressed in aberrant synthesis phase induction. Recent studies have validated this reagent as a marker for cervical dysplasia. Its usefulness in evaluating dysplasia in other tissues has not been extensively studied in the literature. The purpose of this study is to evaluate ProEx C as a marker for detecting and grading dysplasia in Barrett's esophagus, the main risk factor for developing distal esophageal adenocarcinoma.

Design: A retrospective analysis and immunostaining with ProEx C (BD Diagnostics, Burlington) were performed on 48 esophageal biopsy and resection specimens of Barrett's esophagus (17 negative for dysplasia, 12 indefinite, 9 low-grade, 10 high-grade dysplasia). Nuclear staining in the lower two-thirds of the metaplastic glands was considered negative. A positive stain was defined as nuclear staining extending to the upper third and surface epithelial cells (SECs) of the metaplastic glands. The positive stain was further classified as rare, focal strong, or diffuse strong. A statistical analysis was performed.

Results: Results are summarized in the following table:

Diagnosis	Negative	Positive			
	(%)	Rare (%)	Focal Strong (%)	Diffuse Strong (%)	Total (%)
No Dysplasia	15/17 (88)	1/17 (6)	1/17 (6)	0/17 (0)	2/15 (12)
Indefinite	4/12 (33)	5/12 (42)	3/12 (25)	0/12 (0)	8/12 (67)
Low-Grade	3/9 (33)	3/9 (33)	3/9 (33)	0/9 (0)	6/9 (67)
High-Grade	1/10 (10)	1/10 (10)	5/10 (50)	3/10 (30)	9/10 (90)

Using dysplasia (indefinite, low-grade or high-grade) as an endpoint, ProEx C staining extending to the upper third and SECs of metaplastic glands in biopsy proven dysplasia yielded a sensitivity of 74% (23 of 31), a specificity of 88% (8 of 31), a positive predictive value (PPV) of 92% and a negative predictive value (NPV) of 65%. Using high-grade dysplasia as an endpoint yielded a sensitivity of 90% (9 of 10), a specificity of 58% (22 of 38), PPV of 36% and NPV of 96%.

Conclusions: The ProEx C immunohistochemical stain exhibits a high sensitivity (74%), specificity (88%), and PPV (92%) when used to identify and grade dysplasia in cases of Barrett's esophagus. When used in conjunction with routine histological analysis, ProEx C can be a valuable confirmatory test for identifying and grading dysplasia in Barrett's esophagus.

682 Carcinoma of Mullerian Origin Presenting as Colorectal Cancer: Histologic and Immunohistochemical Studies of 13 Cases

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Background: Carcinomas of mullerian origin involving colorectum in women with no history gynecologic malignancies are diagnostically challenging. In this study, we described 13 cases of carcinoma of mullerian origin with clinical presentation mimicking colorectal carcinoma. Salient morphologic features as well as selective immunohistochemical markers that might be useful for differential diagnosis were described.

Design: Thirteen cases of carcinoma of mullerian origin with clinical presentation mimicking colorectal cancer were identified from three large hospitals. None of patients had concurrent or history of gynecologic malignancies 10 years prior to the presentation. H&E slides from each case were reviewed with specific attention to the presence or absence of associated endometriosis. Immunohistochemistry with a panel of markers that may aid in the correct diagnosis were performed.

Results: The average patient age was 63.9 years (range 34-86). All cases except one presented as rectosigmoid or rectovagnal septum mass. The presenting symptoms were rectal bleeding (4/11), rectosigmoid mass (6/11), vaginal mass (1/11), abdominal pain or constipation (2/11). The average size of tumor was 4.2 cm (range 2.4-15.0 cm). Nine of 13 cases had colorectal biopsy and one had vaginal biopsy. The tumor were either moderately differentiated endometrioid (6/11), high grade serous (2/11), mixed serous with endometrioid (2/11), undifferentiated (1/11), or malignant mixed mullerian tumor (2/11). In 8 of 13 cases, endometriosis were identified adjacent or within the tumor. One case had endosalpingiosis. Immunohistochemical stains showed following positive results: CK7(13/13), ER(13/13), PR (10/13), CK20 (0/13), CDX-2(0/13).

Conclusions: Carcinoma of mullerian origin can present as bulky mass in rectosigmoid or rectovaginal septum clinically mimicking primary colorectal cancer. Most of them are of endometrioid or serous types. These results also suggest that endometriosis might be the etiological factor. Identification of benign endometriosis and immunohistochemical stain with a panel of markers (CK7, CK20, CDX2, ER, and PR) is very helpful for the confirmatory diagnosis.

683 Ezrin Overexpression Is Associated with Non-Gastric Location and Inferior Disease-Free Survival in Gastrointestinal Stromal Tumors (GISTs)

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Background: Ezrin, a member of ezrin-radixin-moesin family, acts as a linker protein between the cell membrane and actin cytoskeleton to integrate cell adhesion-mediated signaling. It is known to implicate tumor progression, metastatic dissemination, and adverse outcomes in several cancer types, including pediatric and adult sarcomas. Despite upregulated ezrin recently shown by cDNA expression profiling, no study has systematically evaluated the significance of ezrin expression in a large, well-characterized cohort of GISTs.

Design: Ezrin immunostain was assessable in 347 cases on tissue microarrays of primary GISTs. Among these cases, mutation variants of *KIT* and P*DGFRA* receptor tyrosine kinase (RTK) genes were confirmed in 188 cases by sequencing with or without precedent DHPLC screening and dichotomized into two prognostically different groups. Follow-up was obtained in 313 cases with a median of 57 months. Ezrin overexpression, defined as ≥50% of tumor cells with moderate or strong cytoplasmic staining, was correlated with disease-free survival (DSS), NIH risk level, Ki-67 labeling index (LI), and RTK genotypes, etc.

Results: Ezrin overexpression, present in 66% of GISTs, was significantly associated with the non-gastric location (p= 0.002) and decreased DFS (p=0.032, univariately). However, it was not related to NIH risk category, Ki-67 LI, RTK genotypes, and other variables. In multivariate survival analysis, ezrin overexpression remained independently predictive of adverse outcome (p=0.027, risk ratio [RR]=2.038), together with Ki-67 LI>5% (p<0.001, RR=4.044), high risk category (p<0.001, RR=3.563), and non-gastric location (p=0.006, RR=2.356).

Conclusions: Ezrin is frequently overexpressed in GISTs, especially those arising from non-gastric sites. Although it is independent of NIH risk category, cell proliferation, and RTK genotype, ezrin immunoreactivity represents a valuable prognostic marker in GISTs, suggesting a causative role in conferring an aggressive phenotype.

684 Small Bowel Pathology Revealed by Double Balloon Enteroscopy

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Background: Double balloon enteroscopy is emerging as an important tool for the diagnosis of small bowel lesions not accessible by routine upper endoscopy and colonoscopy. The range of small bowel pathology uncovered by this new technique has not been systematically reported from a U.S. center.

Design: All small bowel biopsies obtained by double balloon enteroscopy (oral or anal approach) were identified by search of our surgical pathology database. The indication for the procedure, enteroscopic findings, and any follow-up procedures were recorded.

Results: Small bowel biopsies from 182 patients (pts) were reviewed. The indications for the procedure were: obscure G.I. bleeding (n = 103), abdominal pain (32), abnormal radiographic study (27), suspected or known Crohn's disease (18), diarrhea & weight loss (17), Peutz Jeghers syndrome (5), and other (15). Polypoid lesions were biopsied in 25 patients: PJ polyp (5), lymphoma (3), heterotopia (3), hemangioma (3), adenoma (2), carcinoid (2), adenocarcinoma (1), other (6). The pts with carcinoid and adenocarcinoma later underwent surgical resection. Of 25 pts with endoscopically evident ulcers 15 were taking NSAIDs and had biopsies c/w NSAID injury, but in 10 pts an etiology was unclear. Two of these 10 pts underwent resection with histology c/w "non-specific chronic small bowel ulcers" as described in Japan (*Gastrointest Endosc 2007; 66:S99*). There was no evidence of celiac disease, ischemia, vasculitis or thrombosis in these cases. In 11 pts with known Crohn's disease biopsies confirmed additional small bowel involvement by Crohn's. In 7 pts with suspected Crohn's biopsies confirmed the diagnosis. In 67 pts only normal mucosa was identifed endoscopically. Biopsies were obtained in 46 of them, all of which revealed no pathologic change. In 15 pts there were subtle changes in mucosal appearance but biopsies were normal in all. Biopsies from 8 pts with scalloped/fissured mucosa revealed celiac disease in 3, NSAID enteritis in 1 & normal mucosa in 4 pts. Submucosal lesions were identified in 9 pts but all biopsies were non-diagnostic. Two of these pts later underwent resection of GISTs.

Conclusions: Small bowel enteroscopy is a useful tool for the diagnosis of clinically important pathology of the jejunum and ileum. Better techniques for biopsy of submucosal lesions are necessary. Biopsy of enteroscopically normal or near normal mucosa has a low diagnostic yield. The etiology of discrete small bowel ulcers identified by this procedure requires further study, as not all appear to be NSAID related.

685 CD61, CD31 and CD34 Improve Diagnostic Accuracy in Gastric Antral Vascular Ectasia and Portal Hypertensive Gastropathy

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Background: Portal hypertensive gastropathy (PHG) and gastric antral vascular ectasia (GAVE) have overlapping clinical, endoscopic and histologic features that may make their differentiation difficult. In addition, correlation between the endoscopic impression of PHG or GAVE and histologic findings is often poor. Correct distinction between these two entities is important since their therapies differ. As a result, we sought to determine whether the use of CD31 and CD34 (vascular markers) or CD61 (to highlight platelet thrombi) could aid in the diagnosis and differentiation of these two disorders.

Design: The pathology database was searched for all cases with a histologic diagnosis of GAVE or PHG. In addition, all biopsies with an endoscopic impression of GAVE or PHG (not histologically confirmed) were included as separate groups. Controls consisted of histologically normal antral biopsies with no endoscopic features of GAVE or PHG. All biopsies were stained with antibodies to CD31, CD34 and CD61. CD31 and CD34 stained sections were digitally scanned and analyzed with the use of Chromavision® software. CD61 staining was graded as present or absent.

Results: The CD61 stain was positive in all 11 (100%) histologically confirmed cases of GAVE, and in 9 of 17 (78%) cases endoscopically felt to be GAVE. CD61 positivity occurred in 2 of 11 (18%) histologically confirmed cases of PHG, and in 4 of 12 (33%) cases of endoscopically suspected PHG. None of the control biopsies exhibited CD61 staining. These differences were statistically significant in all groups (p<.0001). Review of the H&E slides from all PHG (histologic or endoscopic) cases with positive CD61 stains showed other histologic features allowing their reclassification as GAVE. Microvessel densities were significantly higher in GAVE and PHG compared with controls. Microvascular density in histologically confirmed PHG did not differ significantly from endoscopically suspected PHG. Review of the H&E slides from the endoscopically suspected PHG cases showed frequent active gastritis which appeared to obscure recognition of ectatic vessels.

Conclusions: CD61 immunostains reliably differentiate GAVE from PHG. Microvessel densities are significantly higher in both GAVE and PHG compared to normal antrum. In the absence of features other than vascular ectasia, as occurs in PHG, this increased vascular density may be difficult to recognize on routine H&E sections. Vascular markers such as CD31 or CD34 may make identification of aberrant vessels easier in suspected cases of PHG.

686 Utility of Cardia Biopsies in the Distinction between Reflux Esophagitis and Eosinophilic Esophagitis in Pediatric Patients

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Background: Eosinophilic esophagitis (EE) is an increasingly recognized clinicopathologic diagnosis. There is tremendous overlap in clinical and histologic features between EE and gastroesophageal reflux disease (GERD). Differentiation is important as treatment differs between the 2 disorders. According to the latest consensus

guidelines, >15 eosinophils in one HPF is diagnostic of EE in the proper clinical context; however, EE is often patchy and significant overlap in eosinophil counts exist with GERD. In the adult population, a strong correlation between carditis and GERD has been established. In children the presence and significance of gastric cardia and carditis is still being established. The objective of our study was to determine if the presence of carditis could be used to histologically differentiate between GERD and EE in the pediatric population.

Design: 41 patients (pts), 16 months to 18 yrs of age, presenting for upper endoscopy were prospectively enrolled. Two additional biopsies from the gastric cardia straddling the EG junction were obtained in each pt. Three GI pathologists reviewed the biopsies and documented the presence of cardia +/- carditis (defined as neutrophilic inflammation or dense mononuclear cell infiltrate). Cardiac lamina propria eosinophil counts were also performed. Each patient's chart was reviewed to document symptoms, medication history, therapy response, allergic evaluation including RAST and pH probe monitoring. Based on clinicopathologic consensus pts were placed in 1 of 5 categories: Normal, EE, Treated EE, GERD, Co-existent (clear evidence for both EE and GERD).

Results: Cardiac mucosa was successfully obtained in 32 pts with 8 positive for carditis. If carditis was present the patient had GERD and no EE pt had carditis (PPV=100%).

	REFLUX*	EE*	CO-EXISTENT*	NORMAL	
Carditis	7	0	1	0	
Cardia, WDA	0	8	3	13	
					32
Fundic	2	0	2	3	
Squamous	0	2	0	0	
TOTAL	9	10	6	16	41

*P<0.0001

Eosinophils were rare in normal cardiac lamina propria (range 0-1/HPF). Although increased in both, the mean numbers of cardia eosinophils did not significantly differ between EE and GERD pts, while in treated EE pts the count dropped to the normal range (0-1/HPF). Intestinal metaplasia was not identified in any patient.

Conclusions: Biopsies of the gastric cardia can be used to distinguish reflux esophagitis from EE in the pediatric population. Carditis occurs only in GERD or co-existent GERD/EE pts and not in EE alone.

687 Correlation of Expression of the Intestinal Biomarkers Guanylyl Cyclase C and CDX2 in a Large Cohort of Poorly Differentiated Colorectal Carcinomas

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Background: Guanylyl cyclase C (GCC) a receptor for bacterial diarrheagenic enterotoxins is expressed selectively by intestinal mucosal epithelium and is an endogenous downstream target of CDX2. The expression of GCC is preserved throughout the adenoma/carcinoma sequence in the colorectum. Detection of GCC expression by RT-PCR is currently being validated as a technique to detect occult lymph node metastases in patients with colorectal cancer (CRC) and for circulating cells in the blood for postoperative surveillance. Although GCC is widely expressed by well-differentiated CRC its expression in poorly differentiated CRC has not been evaluated.

Design: A tissue microarray was created from 73 archival specimens including 43 poorly differentiated (PDC), 15 undifferentiated or medullary (MC), 12 signet ring cell (SRC) and 3 neuroendocrine (NE) CRCs. Matched normal colonic mucosa was used as a positive control. Immunohistochemical staining for GCC and CDX2 was evaluated as positive or negative based on at least a 10% extent of staining.

Results: Out of the 73 tumor samples 77%, 47%, 83% and 67% of the PDC, MC, SRC and NE tumors were positive for GCC and 77%, 40%, 83% and 100% of these subsets were positive for CDX2. There was excellent correlation between GCC and CDX2 expression ($p<0.001$). Immunopositivity for GCC was greater than 95% in a separately stained microarray series of well/moderately differentiated CRCs.

Conclusions: In conclusion, GCC expression is lost in almost a quarter of poorly differentiated and half of undifferentiated CRC. Therefore the utility of GCC as a diagnostic and prognostic marker for CRC may be questionable in poorly differentiated colorectal neoplasms.

688 CpG Island Methylation in Serrated and Conventional CRC Precursor Neoplasms: How Individual Marker Genes and Panels Relate to Histology and Oncogene Mutation Status

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Background: Epigenetic silencing of suppressor and mutator genes by CpG island methylation (CIM) has been studied mainly in advanced colorectal cancer (CRC). Here we investigate the relationship of CIM to histology and oncogene status in CRC precursor polyps.

Design: DNA samples from 50 polyps, 10 BRAF mut sessile serrated adenomas, 30 dysplastic serrated polyps (SA-14 KRAS mut and 16 BRAF mut) and 10 KRAS mut conventional adenomas(CoA) were assayed by MS-PCR for the following 15 CIM markers: p16, hMLH1, MGMT, MINT1, MINT2, SOCS1, Neurog1, RUNX3, IGF2, CACNA11G, SFRPS, RASSF2A, Reprimo, 30ST2 and HPP1. These included the canonical panel (Cn), that proposed by Weisenberger et al.(Wr) and methylation markers linked to KRAS mut by Nagasaka et al.(Na). Groups were compared using Fisher Exact and U-Mann Whitney tests as appropriate.

Results: All 15 markers showed higher (11) or equivalent (4) positive frequency rate among BRAF vs KRAS mutated categories; the mean BRAF/KRAS marker positivity ratio was 2.75, 1.5 and 1.37 respectively. The specificity of individual genes for either group was low. All 3 panels showed significant differences in positive marker frequency rate for BRAF mut compared to KRAS mut precursors (Wr: $p<0.0001$; Na: $p=.001$; Cn: $p=.014$) and for KRAS mut SA compared to KRAS mut CoA (Wr: $p=.07$; Na: $p=.02$; Cn: $p=.02$).

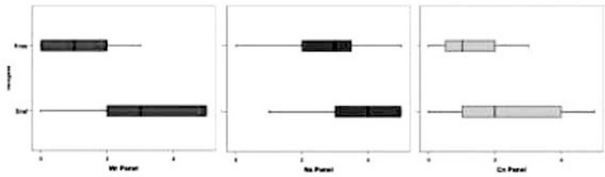

Fig 1 Legend: Box-plots comparing the distribution of marker positivity frequency among KRAS mut versus BRAF mut CRC precursors using 3 separate panels, from left to right Wr, Na and Cn.

Conclusions: A series of gene methylation markers comprising 3 distinct panels indicated, with varying precision, that BRAF mut vs KRAS mut and serrated vs non-serrated histology in CRC precursor neoplasms were associated with higher levels of CpG island methylation.

689 Routine Mismatch Repair Protein Immunostaining in Colorectal Carcinomas – Equivocal MSH6 Staining Is a Challenge

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Background: Over the last 2 years, we have routinely performed mismatch repair protein (MMR) immunohistochemistry on all colorectal cancer (CRC) resections. While there have been no equivocal MLH1, MSH2 or PMS2 stains, MSH6 has been equivocal in some cases. To determine whether these cases were likely due to Lynch syndrome or another cause, we evaluated all MSH6-equivocal and absent cases.

Design: CRC resections with equivocal or absent MSH6 expression were obtained from our database. Age, family history (FH), tumor site and neoadjuvant therapy (NT) were noted. H&E slides were evaluated for amount of tumor and histologic features of microsatellite instability (histMSI), including mucin and/or Crohn's-like reaction. Initial and repeat MSH6 and MLH1, MSH2 and PMS2 slides were reviewed. MSH6 was scored as present (≥5% convincing nuclear staining), equivocal (1-5% or faint staining) or absent. Cases were reviewed by 3 GI pathologists and contained appropriate controls. Adjacent stroma and/or normal colon served as an internal control.

Results: Absent (8) or equivocal (5) MSH6 expression was seen in 13 of 306 cases. Six of the 13 (3 rectum, 3 right) with ample tumor showed absent MSH6. No patient (age 48-54) had FH of CRC, one had prior NT, and 5 tumors showed histMSI. Mutational analysis, completed in 1, demonstrated *MSH6* mutation. The 7th case occurred in the rectum of a 32-year-old with FH of CRC in a distant relative. The tumor did not show histMSI but was MSH6-absent; however, tumor was limited (prior NT). An additional MSH6-absent rectal tumor occurred in a 78-year-old with no FH of CRC and with little evaluable tumor (prior NT). MSH6 was present in the pre-treatment biopsy; mutational analysis confirmed lack of *MSH6* mutation. The final 5 cases (patients age 50-67) had equivocal MSH6; and 1 had FH of CRC while another had histMSI. Four had NT, 3 with little tumor in the resection. All 5 are likely MSH6-present. Mutational analysis has been completed in 2 confirming the lack of *MSH6* mutation.

Conclusions: Routine immunohistochemical MMR protein evaluation in CRC is relatively straightfoward in most cases; equivocal results are uncommon, but MSH6 interpretation is difficult in occasional cases. Before molecular workup is undertaken in MSH6 equivocal/negative tumors, age, FH, histMSI, NT and sufficient tumor should be considered. Repeat MSH6 on a prior biopsy may be helpful. Studies are necessary to evaluate potential causes of difficulty in MSH6 interpretation including antibody and fixation issues.

690 Differential Immunoexpression Profiles in Appendiceal Mucinous Neoplasm

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Background: There has been a lot of debate on appendiceal mucinous neoplasm. Recently, appendiceal mucinous neoplasm of uncertain malignant potential (UMP) has been introduced. UMP is defined as follows; appendiceal mucinous neoplasm of bland cytoarchitecture, showing (1) epithelium deeply into underlying tissue, or (2) cystic gland-like structure in wall, or (3) uncertainity of complete excision, in the condition of loss of musularis mucosae and unclear invasion. The present study shows that characteristic protein expression profiling would be helpful to classify appendiceal mucinous tumor.

Design: Total 70 cases of appendiceal mucinous neoplasm were histologically classified into three groups; 34 mucinous adenoma (BENIGN), 21 UMP, and 15 mucinous adenocarcinoma (MALIGNANT). MALGINANT contained appendiceal mucinous neoplasm with low grade cytoarchitectural atypia (so-called low grade malignant potential) and high grade atypia. Iimmunohistochemistry was performed for 24 antibodies; oncogenic function proteins (bcl-2, beta-catenin, CEA, C-erbB2, c-kit, Cox-2, Cyclin D1, EGFR, Ki 67, NF-kappaB, VEGF), tumor suppressors (E-cadherin, FHIT, hMLH1, p53, p63, smad4), cell cycle regulators (p21, p27, p16) and mucin proteins (MUC1, MUC2, MUC5AC, MUC6). As controls, 32 non-neoplastic appendiceal tissues were included.

Results: Among 24 examined proteins, nine proteins were more frequently altered in MALIGNANT than BENIGN ($p < 0.05$); beta-catenin (10% in BENIGN *vs.* 47% in MALIGNANT), CyclinD1 (43% *vs.* 93%), NF-kappaB (13% *vs.* 60%), VEGF (15% *vs.* 53%), E-cadherin (0% *vs.* 33%), p53 (9% *vs.* 47%), MUC2 (58% *vs.* 100%), MUC5AC (23% *vs.* 93%), and high Ki-67 labeling (31% *vs.* 67%). The expression profiles of

these nine proteins in UMP were put in the middle of BENIGN and MALIGNANT. Accordingly, immunoexpression profiles of UMP were distinct from those of BENIGN or MALIGNANT ($p < 0.05$). Besides, BENIGN, UMP and MALIGNANT were different in the number of aberrant proteins ($p < 0.05$); mean 2.06 vs. 3.78 vs. 5.27 out of nine proteins, respectively.

Conclusions: Appendiceal mucinous neoplasm can be classified into three groups, and mucinous neoplasm of uncertain malignant potential is a distinct entity in terms of molecular markers. Immunoexpression profiling may be useful to predict the clinicopathogical behavior of UMP.

691 The Potential Role of p27, Skp2, and PTEN Expression in Gastric Carcinoma

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Background: $p27^{Kip1}$ is a cell-cycle inhibitory protein and its downregulation is mediated by its specific ubiqitin subunit Skp2. PTEN is a tumor suppressor gene which upregulates p27. This study investigates p27, Skp2 and PTEN expression in gastric carcinoma (GC).

Design: The study included 127 patients with GC who underwent gastrectomy. None of the patients received any chemotherapy or radiotherapy prior to, or after surgery. Eight tumors were TNM-stage I, 39 II, 61 III, and 19 IV, whereas 95 tumors were low-grade (grade I and II intestinal type adenocarcinomas) and 32 high-grade (grade III intestinal type, and diffuse type adenocarcinomas). Formally fixed, paraffin- embedded 4μm sections were subjected to immunohistochemistry using monoclonal antibodies for p27, Skp2 and PTEN. Nuclear staining was considered as positive. Results were correlated with pathologic data and patients' survival. Mean follow-up time was 45.3 months (range 3.5-140 months).

Results: Expression of p27, Skp2 and PTEN was recorded in 78/127 (61.4%), 57/127 (44.8%) and 99/127 (77.9%), respectively. PTEN and p27 expression was higher and Skp2 expression was lower in tumors of early stage and low grade compared to those of advanced stage and high grade. Skp2 expression levels were inversely correlated to p27 and PTEN in gastric carcinomas (p=0.0039 and p=0.0068 respectively). Statistical analysis revealed a positive correlation between PTEN expression and survival (p=0.007); Skp2 expression was negatively associated with survival (p=0.015). Cox regression analysis revealed that tumor grade and stage, and PTEN expression were independent prognostic factors (CI: 0.032-0.502, p=0.03, CI:1.167-5.408, p=0.019, CI: 1.065-41.082, p=0.032, respectively).

Conclusions: The study demonstrates that in GC loss of PTEN and p27 expression and enhancement of Skp2 expression are associated with adverse pathological parameters and increased risk for tumor recurrence. Loss of p27 in gastric carcinomas may be mediated by Skp2 overexpression. PTEN is possibly involved in the regulation of p27 levels via negative regulation of Skp2.

PTEN, p27 and Skp2 expression

	PTEN	p27	Skp2
Low grade GC	87.3±11.9[a]	79.2±13.2[c]	22.41±6.3[e]
High grade GC	7.4±2.5[a]	5.3±3.1[c]	38.85±5.63[e]
TNM stages I+II	86.1±8.34[b]	73.1±7.32[d]	22.78±6.92[f]
TNM stages III+IV	12.4±1.2[b]	5.2±2.7[d]	53.01±7.34[f]

[a, d, f]:p<0.001, [b]: p=0.0029, [c]:p=0.0012, [e]: p=0.031

692 Histologic Mimics of Menetrier's Disease

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Background: Menetrier Disease (MD), a hypertrophic gastropathy characterized by giant rugal folds due to foveolar hyperplasia, with loss of parietal cells, hypochlorhydria, and protein loss, is rarely seen by most pathologists. The goal of this study was to characterize histologic mimics of MD submitted for evaluation for enrollment into a clinical trial for treatment of MD, to determine pitfalls in the diagnosis of this rare disorder.

Design: Material from 27 subjects referred for entry into a clinical trial for treatment of MD was reviewed. Pre-treatment stomach biopsies were evaluated for foveolar hyperplasia, hypermucinous change, dilatation of glands, lymphocytic gastritis; lamina propria eosinophils, plasma cells, edema, smooth muscle, granulation tissue, and presence of microorganisms.

Results: Nine of 27 subjects (33%) referred were considered to have MD. An additional adult patient was diagnosed with CMV-related MD and did not receive treatment. Foveolar hyperplasia, hypermucinous change, dilatation of glands and lamina propria smooth muscle hyperplasia were present in all patients with MD. More than half had prominent lamina propria eosinophils (5/9), and/or plasma cells (6/9), and lamina propria edema (8/9). Lymphocytic gastritis was not a feature of our MD cases. Antral biopsies were available on 2 MD patients which revealed similar changes to the fundic biopsies. Mimickers of MD included gastric involvement by juvenile polyposis (3), Cronkhite-Canada syndrome (1), proton pump inhibitor effect (1), gastric antral vascular ectasia (1), and chronic atrophic gastritis with hyperplastic polyps (7). While hyperplastic polyps and juvenile polyps were characterized by foveolar hyperplasia and hypermucinous change, lamina propria smooth muscle fibers were less prominent than in MD. For 4 patients, hypertrophic changes potentially representing MD were present, but diagnosis was inconclusive due to lack of diffuse gastric body involvement, lack of hypochlorhydria, and/or atypical clinical symptoms.

Conclusions: Foveolar hyperplasia, hypermucinous change, dilatation of glands and lamina propria eosinophilia, plasma cells, edema, and smooth muscle hyperplasia are a constellation of features characteristic of MD, but correlation with endoscopic and clinical findings remains essential for accurate diagnosis. Hyperplastic polyps and previously undiagnosed juvenile polyposis were the most common lesions confused with MD.

Genitourinary

693 IFI16: A Novel Growth Suppressor in Human Prostate Cancer

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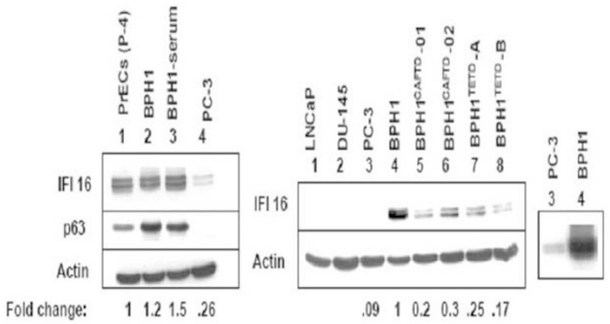
Background: Tumor suppressors (TS) provided a molecular basis for multistep carcinogenesis; human cancers have multiple genetic alterations including loss of TS. Interferon inducible IFI16 (encoded by *IFI16* gene) is a human member of the p200-protein family and its increased expression in human normal prostate epithelial cells (PrECs) leads to cellular senescence-associated cell growth arrest. Our aim is to study the role of IFI16 protein in PC, as studies of TMPRSS2 overexpression predict aggressive PC.

Design: By immunoblotting, expression of IFI16 protein was compared in total cell lysates from cultured normal PrEC, a benign prostate hyperplasia cell line (BPH-1), and derivatives from the BPH1 cell line that have acquired the ability to form tumors in nude mice (aggressive lines), and PC cell lines (RWPE-1, DU-145, PC-3 and LNCaP). Human prostate tissue array slides (Imgenex) containing 98 prostate samples and 30 of our own archival cases (total 45 BPH, 29 Gleason Score(GS)>6, 26 GS7, 24 GS<8, and 4 metastatic PC to bone marrow and lymph nodes) were stained with H&E and IHC for IFI16 (goat polyclonal sc-6050 Santa Cruz Biotech) and p63 (Ventana pre-dilute). Two pathologists independently reviewed and concurred on GS and IHC intensity and percentage of glands staining for IFI16 on a scale of 0 (no stain) to 2 (highest). Expression of p63 was used as a control in cell cultures and tissue IHC.

Results: Normal PrEC and BPH cell lines expressed IFI16 protein; however, IFI16 is reduced or lost in more aggressive BPH1 and most human PC cell lines tested. P63 was lost in all PC cells and tissue, as a control.



Loss of IFI16 protein expression is associated with the development of an aggressive form of prostate cancer

IHC expressed IFI16 weakly in BPH, and strongly in low grade PC; most high grade PC and metastasis lost the IFI16 staining. P=0.0003.

STAIN	0	1	2
BPH	22(49%)	23(51%)	0
GS<6	0	11(38%)	18(62%)
GS7	2(7%)	15(57%)	9(34%)
GS<8	13(54%)	9(37%)	2(8%)
METASTASES	4(100%)		

IHC for IFI16 – number of cases (% of cases)

Conclusions: This study shows molecular and phenotypic evidence that IFI16 loss may serve as a late hit in the multistep carcinogenesis of PC. Our model shows loss of IFI16 expression in higher grade/stage PC; this may predict aggressive PC, similar to TMPRSS2 overexpression. Further studies are warranted to confirm our findings.

694 PCA3: A Urine-Based Genetic Assay for Detection of Prostate Cancer in Men with Elevated PSA

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Background: Serum PSA has been labeled the most important tumor marker in oncology and is a valuable screening tool for prostate cancer. However, since it is not prostate-cancer specific, there is no threshold that separates men with and without cancer with a high level of accuracy. We investigated the value of PCA3 in predicting the likelihood of prostate cancer.

Design: We undertook a prospective, multi-practice, community-urologist-based, and IRB approved clinical trial to evaluate PCA3. Urine samples were obtained from 974 men with elevated serum PSA (≥2.5ng/ml) and/or abnormal digital rectal examination prior to routine 10-core prostate biopsy following standard study protocol in 30 medical practices. Urine samples were processed within 48 hours of collection. PCA3 and PSA mRNA were isolated, amplified and quantified by magnetic target capture, transcription-mediated amplification, and chemiluminescent hybridization protection assay technologies. The PCA3 value was determined using the ratio of PCA3 mRNA copy number to the PSA mRNA copy number multiplied by 1,000.

Results: In total, 380 of 974 patients (39%) were diagnosed with prostate cancer, with a mean Gleason score of 7 (range, 6-9) and 26% (range 1-100%) of specimen involvement by cancer. An additional 106 cases (11%) had only high-grade PIN and/or atypical small