due to chronic androgen deficiency or to the genetic abnormality per se remains to be elucidated. Molecular genetic studies are required to obtain deeper insight into the pathogenesis of pituitary tumors in patients with this disorder.

### 428 CEAMCAM1 Is Expressed by Human Thyroid Carcinoma Cells and Represents a Target for Vitamin $D_3$ Therapy

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**Background:** CEACAM1, formerly known as biliary glycoprotein, CD66a, C-CAM1 and pp120, is a member of the CEA immunoglobulin superfamily. CEACAM1 is a putative tumor suppressor based on its diminished expression in some tumors including colon carcinoma. However, CEACAM1 is also over-expressed in tumors such as non-small cell lung cancer. The role of CEACAM1 in thyroid tissue remains unknown.

**Design:** In the present study we investigated the expression of CEACAM1 in thyroid tissue and tumor cell lines, and its relationship to thyroid cell growth and adhesion. Since Vitamin D (VD) and its analogues, EB1089 and KH1060 are known to inhibit thyroid cancer cell growth, we examined the effect of these compounds on the expression of CEACAM1 in thyroid cancer.

**Results:** Using a tissue array of primary human thyroid samples, CEACAM1 was identified in a subset of papillary carcinomas that had lymph node metastases and in poorly-differentiated and anaplastic carcinomas. There is a marked apical luminal localization of CEACAM1 in differentiated thyroid carcinomas that is dysregulated in dedifferentiated malignancies. No expression was found in normal thyroid tissue or benign tumors however there was focal staining in atypical cells of chronic lymphocytic thyroiditis. CEACAM1 is expressed by the aggressive ARO (anaplastic) and MRO (follicular) carcinoma cell lines but not in the papillary carcinoma cell lines TPC-1 and NPA as determined by multiple techniques. Treatment of ARO and MRO cells with VD and its analogues, EB1089 and KH1060, significantly decreased CEACAM1 levels, reduced S-phase entry, and up-regulated p27 protein accumulation.

**Conclusions:** These findings suggest that CEACAM1 is expressed in a more aggressive group of thyroid malignancies where it may play a role in architecture, stromal interactions and metastasis. It represents a target for VD therapy which modulates its expression and may have potential therapeutic applications.

### 429 Significance of GLUT-1 Expression in Thyroid Gland Tumors: Its Availability for Discrimination between Papillary Carcinoma and Follicular Carcinoma

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**Background:** Glucose transporter-1 (GLUT-1), one of GLUT isoforms, has been demonstrated to play a role in facilitating effective transportation of glucose. It is well known that GLUT-1 expression is associated with an increased uptake of glucose during malignant transformation. The purpose of this study focused on clarifying whether GLUT-1 would be useful in discriminating between papillary carcinoma and follicular carcinoma and whether it would be a biological marker associated with a tumor development.

**Design:** Materials: Two hundred and sixty-five thyroidectomy specimens used were composed as follows: papillary carcinoma, 126 cases; follicular carcinoma, 60 cases; follicular adenoma, 57 cases; adenomatous goiter, 22 cases. Tissue blocks were routinely processed for histological diagnosis. Methods: Immunohistochemical expression GLUT-1 (DAKO, polyclonal, rabbit, diluted at 1:50) was studied using an indirect peroxidase method. More than 10% of immunoreaction was regarded as positive.

**Results:** GLUT-1 expression was observed in 71% (89/126) of papillary carcinomas and 5% (3/60) of follicular carcinomas. Follicular adenomas and adenomatous goiters failed to show positive reaction. Strong expression on the cell membrane was limited to 24 cases of papillary carcinoma. Among them, 92% (22/24) developed the metastatic disease in the regional lymph nodes. There was no tendency that the expression was strengthened according to an increase of tumor size.

**Conclusions:** GLUT-1 is considered to be related to a tumor development of papillary carcinoma and is also helpful in discriminating beween papillary carcinomas and follicular carcinomas. GLUT-1 overexpression on the cell membrane may demonstrate an aggressive biological behavior of papillary carcinomas, resulting in lymph node metastasis.

### 430 Stromal Cell-Derived Factor-1 Expression in Malignant Thyroid Neoplasms of Follicular Cell Origin and in Atypical Foci in Hashimoto's Thyroiditis

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**Background:** The alpha-chemokine stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 have been recognized for their roles in stem cell trafficking, and recent data suggests that the CXCR4/SDF-1 pathway may also be important for regulating tumor metastasis. Little information exists regarding the role of SDF-1 in thyroid neoplasia.

**Design:** Formalin-fixed paraffin-embedded recut sections of 16 normal thyroid glands, 15 multinodular goiters, 11 cases of Hashimoto's thyroiditis, 14 follicular adenomas, 2 follicular carcinomas, 16 papillary carcinomas, and 2 anaplastic carcinomas were immunostained for SDF-1 (1:25, R&D Systems) using standard avidin-biotin techniques. As appropriate for the process, the percentage of tumor cells or non-neoplastic follicular cells that demonstrated immunoreactivity for SDF-1 was scored as follows: 0=<10%, 1=10-50%, 2=>50%. A positive stain was defined as 10% or more immunoreactivity. Staining intensity was graded as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong).

**Results:** SDF-1 staining was observed in 12/16 papillary carcinomas, 1/2 follicular carcinomas, and 2/2 anaplastic carcinomas, while only 1/45 benign processes, a partially infarcted follicular adenoma, demonstrated immunoreactivity. 8/11 examples of Hashimoto's thyroiditis showed SDF-1 expression in atypical microscopic foci consisting of cells with incomplete morphologic features of papillary carcinoma and/or Hurthle cell-like features, in most cases accounting for 10-20% of follicular cells in the section. Statistical analysis of SDF-1 staining in malignant neoplasms of follicular cell origin revealed sensitivity=75.0%, specificity=97.8%, PPV=93.8%, NPV= 89.8%.

**Conclusions:** SDF-1 is a highly specific marker of malignant neoplasms of follicular cell origin that may be useful for supporting a malignant interpretation. Focal SDF-1 staining, however, should be interpreted with caution in a setting of Hashimoto's thyroiditis. The expression of SDF-1 in Hashimoto's thyroiditis correlates with abnormal histopathologic features and parallels published descriptions of RET immunoreactivity in atypical foci in Hashimoto's thyroiditis *(Am J Pathol 2002,160:2157-2167)*. The relationship of these foci to thyroid carcinogenesis merits further investigation.

# Gastrointestinal

### 431 Genetic Interactions and Histologic Associations of NOD2, DLG5, OCTN1 and OCTN2 Polymorphisms in Ileal Crohn's Disease

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**Background:** Crohn's Disease (CD) develops as a result of an inappropriate immune response to normal bacterial flora of the intestine. To date, four genes (DLG5, NOD2, OCTN1 and OCTN2) that harbor relatively common polymorphisms have been implicated in both familial and sporadic CD. Linkage studies have implicated seven separate alleles in these four separate genes as major genetic determinants of CD. Little is known about the genetic interactions between these genes or the histologic manifestations of CD in patient's who carry these alleles.

**Design:** 82 cases of ileal CD requiring surgical resection were identified. DNA was retrieved from archival, parrafin-embedded tissue from these cases and was genotyped for the R30Q DLG5, P1371Q DLG5, R908G NOD2, L503F OCTN1 and -G207C OCTN2 polymorphisms in multiplex format using Sequenom technology. Disease-associated alleles were correlated with the histologic features.

**Results:** Disease-associated alleles were present in 67/82 (81.7%) of patients. 100% of patients carrying the G908R NOD2 risk (4/82, 3.8% carrier frequency of G908R NOD2) allele were also carriers of OCTN1 and OCTN2 alleles, compared to only 36/78 (46.1%) of patients without the G908R NOD2 allele (P<0.05). No association was identified between DLG5 and either the G908R NOD2 allele or the OCTN genes in ileal CD. Ulceration was significantly more frequent in homozygous (3/3; 100%) and heterozygous (12/20; 60%) carriers of DLG5 risk alleles compared to non-carriers (8/65; 12.3%; P<0.001). No association was identified between any of the risk alleles and granuloma formation or with any of the other histologic features evaluated.

**Conclusions:** These results suggest that risk alleles may play a role in a substantial proportion of CD patients and that a genetic interaction exists between the OCTN1 and OCTN2 genes and NOD2. Ulceration may be frequent in patients carrying a risk allele for DLG5.

### 432 Role of Leukocyte $\beta_2$ Integrens in Pathophysiology of Colitis

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**Background:** Inflammatory bowel diseases (IBD), are chronic inflammatory disorders whose etiology remains unknown. Several reports have suggested that infiltration of leukocytes into intestinal tissue is a pathognomonic hallmark for this disease. Pathological features of colitis are inflammatory adhesion molecule up-regulation, increased leukocyte recruitment, and increased oxidative stress in the colon. The leukocyte $\beta_2$ integrins are heterodimetric adhesion molecules that participate in immune cell adhesion that are exclusively expressed on leukocytes. They consist of a common CD18 β-chain that is non-covalently linked to one of four α -chains termed CD11a (LFA-1), CD11b (Mac-1), and CD11c (p150), and CD11d. In this study, we examined the pathophysiological role of the $\beta_2$ integrins CD18, CD11a, and CD11b in the pathogenesis of experimental colitis.

**Design:** A total of 24 various gene targeted deficient mice 10-12 weeks of age were used for this study. The study groups included CD18 null (n=6), CD11b Null (n=6), CD11a Null (n=6), and wild type (n=6) mice. 3% Dextran Soduim Sulphate (DSS) dissolved in the drinking water was used to induce experimental colitis. Daily assessment of clinical disease activity was measured utilizing the following parameters: stool consistency, weight loss, stool occult blood, and gross rectal bleeding. Three independent histopathological parameters were measured including severity of inflammation, surface epithelial/ crypt damage, and depth of injury of the distal and proximal segments of the colon

**Results:** Our study demonstrates that the leukocyte $\beta_2$ integrins play an important role in the development of inflammatory colitis. The CD18 null mice and CD11a null mice had statistically significant lower cumulative pathological scores of 0.333± 0.333 and 5.92±1.03 respectively versus 22.8±4.73 in wild type mice, which indicates attenuation

of the inflammatory histopathological damage induced by DSS consumption. The CD18 null mutant mice had stastically significant lower clinical disease activity index (DAI) than the wild type mice. Three CD18 null mice had an index of 0.0± 0.0 and the other three had an index that ranged from 0.5±0.3184 to 2.167±0.215. In contrast, the wild type mice had a DAI that ranged from 0.160±0.135 to 3.100 ±0.422.
**Conclusions:** This study demonstrates that genetic loss of CD18 or CD11a is protective during experimental colitis. These results suggest that inhibition of leukocyte adhesion molecules may provide a novel therapeutic approach for inflammatory bowel disease.

### 433 Intestinal Spirochestosis Can Persist for Years and Is Associated with an Increased Prevalence of Abdominal Symptoms and Unexplained Diarrhea

*SC Abraham, R Kannangai, M Torbenson.* Mayo Clinic, Rochester, MN; Johns Hopkins University, Baltimore, MD.
**Background:** Human intestinal spirochetosis (IS) results from colonization of colonic epithelium by Brachyspira species, including B. aalborgi and B. pilosicoli. It is debated whether IS is a cause of abdominal complaints such as diarrhea, pain, and rectal bleeding, or whether it is simply a commensal organism.
**Design:** We analyzed the epidemiologic and pathologic features (age, gender, presence of HIV infection, diarrhea, abdominal pain/bloating, rectal bleeding, duration of infection, and response to antibiotics) in 44 patients with IS confirmed by Warthin-Starry stain. Data were compared to a control group of 220 consecutive patients without IS who underwent colonoscopic biopsies, using chi-square analysis and t-test. Genotype analysis was also performed in 35 IS cases using PCR primers specific for B. aalborgi and B. pilosicoli 16S rRNA.
**Results:** As compared to controls, patients with IS were younger (IS: mean age 52.7 yrs, range 12-82 yrs; controls: mean 59.5 yrs, range 4-85 yrs; p<0.02) and were more likely to suffer from unexplained diarrhea (34% vs 17%, p=0.01), other abdominal complaints such as pain/cramps/bloating (41% vs 23%, p=0.01), and HIV infection (4.5% vs 0%, p<0.01). There were no significant differences in gender (55%M vs 48%M, p=0.40), rectal bleeding (14% vs 16%, p=0.70), colonic diverticula (25% vs 31%, p=0.43), or history of chronic inflammatory bowel disease (IBD) (14% vs 13%, p=0.87). B. aalborgi was responsible for IS in 31 of 35 (89%) patients and B. pilosicoli in 3 (9%); there was no significant correlation with symptoms. Seven patients with IS had prior or subsequent colonic biopsies and infection with untreated IS persisted in 5 (71%) at lengths of 3 mo, 3 yrs, 3 yrs, 4 yrs, and 6 yrs. In 4 cases of treated IS (metronidazole) with clinical follow-up, abdominal symptoms greatly improved in 2 (50%) and initially improved but then recurred in the others (50%).
**Conclusions:** B. aalborgi accounts for a majority (89%) of IS in the United States. Untreated IS can persist for years and is significantly more common in patients suffering from clinically unexplained diarrhea and abdominal symptoms including pain/bloating. It is not associated with increased frequencies of rectal bleeding, diverticulosis, or IBD. Clinical follow-up of treated IS in our series was limited, but improvement in symptoms was demonstrated in half (2 of 4) and underscores that IS may be responsible for abdominal symptoms in some patients, rather than simply a commensal.

### 434 Colorectal Carcinoma in Iraq: Clinicopathological Study

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**Background:** Colorectal cancer is the third most common visceral cancer in the United States and the third leading cause of death from cancer in both males and females, in Iraq colorectal cancer is much less frequent.
**Design:** During the period between 1965-2000 802 patients with colorectal malignancies were seen. All the relevant clinical features were recorded. All patients were operated on at the Medical City teaching hospital and two private hospitals (Alousi and Mustansiria). Staging was done according to Astler and Coller staging system. The specimens included, segmental, hemi, subtotal, total colectomy or abdomino-perineal resection specimens.
**Results:** In this study 802 patients with colorectal cancer seen during the period between 1965-2000 were included, 774 (95.6%) were carcinoma. The peak age was between 50-60 years. It was seen in 36% of cases in patients younger than 50 years of age. The male to female ratio was 1.4:1. Multiple colorectal carcinomas were seen in 2.2% of all cases. Arabs were slightly more frequently affected than Kurds.
The predisposing lesions including adenomatous polyps, ulcerative colitis, and familial polyposis were seen in 7.2% of cases. Sigmoid colon and caecum were the most common sites involved in colonic carcinoma. The most common gross appearance in colonic carcinoma was infiltrative lesion while in rectal carcinoma ulcerative lesion was the most common.
The majority of colorectal carcinoma were moderately differentiated followed by poorly differentiated and well differentiated. Most of patients had advanced tumor at the time of diagnosis (Astler and Coller stage C and D). Mucinous and signet ring adenocarcinoma were seen in 11.6% of all cases, affecting relatively young patients.
**Conclusions:** The peak age was between 50-60 years. The male to female ratio was 1.4:1. Arabs were slightly more frequently affected than Kurds. Sigmoid colon and caecum were the most common sites involved in colonic carcinoma. Most of patients had advanced tumor at the time of diagnosis (Astler and Coller stage C and D)

### 435 Cox-2 Expression in Microsatellite Unstable Colorectal Cancer. Differences Regarding MLH1 and MSH2 Loss of Expression

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**Background:** Since the discovery of cyclo-oxygenase 2 (Cox-2) and the introduction of specific Cox-2 inhibitors for the prevention and/or treatment of colorectal carcinoma (CRC), evaluation of Cox-2 expression in this neoplasia may be clinically relevant. Cox-2 overexpression has been detected in 90% of CRC and it seems to be lower in tumors with microsatellite instability (MSI). Some clinicopathological features have been related with MSI phenotype, especially in tumors with MLH1 loss of expression, while CRC with loss of MSH2 expression present similar clinicopathological features to microsatellite stable (MSS) tumors. Differences in Cox-2 expression in both groups may be important in optimal patient selection for Cox-2 inhibition therapy.
**Design:** Formalin-fixed, paraffin-embedded tissues from 67 MSI tumors and 154 MSS tumors were stained with Cox-2 (Oxford Biomedical Research). Staining was scored for intensity (0, 1+, 2+ or 3+) and percentage of staining in malignant cells (1=0-25%, 2=26-50%, 3=51-75%, and 4=76-100%). The sum of the intensity and percentage score was used as the final score (0-2 negative and 3-7 positive). Clinicopathological features were evaluated (age, sex, location, histological type, grade, and TNM stage).
**Results:** Cox-2 was positive in 35/67 (52.2%) of MSI tumors and in 127/153 (83%) of MSS tumors (p<0.000). Positivity was found in 27/56 (48.2%) of MLH1 loss of expression tumors and in 9/11 (81.8%) of MSH2 loss of expression tumors (p=0.041). The relation of Cox-2 expression and clinicopathological features were analysed. We found significant differences between positive expression regarding to location (Right 64.6% vs. left 81.1%; p=0.007), grade (well differenciated 81.5% vs. poorly differentiated 35.3%; p=0.000), and histological type (conventional 82.3%, medullary 12.5%, mucinous 69.6%, others 28.6%; p=0.000). No differences were found in relation with age, sex, margin or TNM stage.
**Conclusions:** MSI CRC with MLH1 loss of expression show lower Cox-2 expression than those with MSH2 loss of expression. However, percentage of positive cases is similar in MSS and MSH2 loss of expression tumors. Therefore, the benefit of Cox-2 inhibitory therapy in MSI CRC could be related to loss of MLH1 or MSH2 expression.

### 436 Expression of Epidermal Growth Factor Receptor in Squamous Cell Carcinomas of the Anal Canal

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**Background:** Immunohistochemical detection of expression of the epidermal growth factor receptor (EGFR) has been used to identify eligible patients with solid malignant tumors, including colorectal adenocarcinoma, for monoclonal antibody treatment (e.g., cetuximab). Whether EGFR is expressed in squamous cell carcinoma of the anal canal, an uncommon malignancy traditionally treated with chemoradiation, has not been investigated.
**Design:** A total of 33 primary squamous cell carcinomas of the anal canal were included in this study. Tumors arising from perianal skin were excluded. Formalin-fixed paraffin-embedded tissue sections were immunohistochemically stained using a monoclonal anti-EGFR antibody (Zymed). The staining was interpreted according to the criteria recommended for colorectal adenocarcinoma. That is, a tumor was considered positive when there was complete or incomplete circumferential membranous staining (with or without cytoplasmic staining) above background level in ≥1% of the tumor cells. The staining intensity was stratified as 1+, 2+ or 3+.
**Results:** A variable degree of EGFR immunoreactivity was observed in 20 (61%) squamous cell carcinomas of the anal canal, among which 13 (65%) exhibited a 2+ or 3+ staining pattern (Table). The staining was diffuse (>50% of the tumor cells stained) in 8 cases (40%), and focal (<50% of the tumor cells stained) in the remaining cases. In only 2 cases, both scored 1+, the immunoreactivity was observed in <5% of the tumor cells. There was no statistical difference among different tumor stages, between keratinizing and non-keratinizing variants, or among tissue blocks with different storage time.

| | Negative | 1+ | 2+ | 3+ | Total |
|---|---|---|---|---|---|
| EGFR | 13 | 7 | 3 | 10 | 33 |

**Conclusions:** EGFR is expressed in more than half of the squamous cell carcinomas of the anal canal. These findings may have important therapeutic implications since targeted therapies have shown promise for other types of malignancy.

### 437 The Clinical Significance of So-Called "Backwash Ileitis" (BWI) in Total Proctocolectomy Specimens (TPC) in Patients with Ulcerative Colitis (UC) Treated with Ileal Pouch-Anal Anastomosis (IPAA)

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**Background:** The concept of "BWI" is confusing to pathologists, gastroenterologists and colorectal surgeons. Although much has been written about BWI, there is still no uniform definition of BWI, nor is it known whether the presence of BWI in a TPC for UC is predictive of an adverse clinical outcome in patients who have been treated with an IPAA.
**Design:** From the database maintained in the Dept of Colorectal Surgery, we identified 92 pts who were reported to have BWI with pancolitis in their TPC. Matched controls (matched for age, gender, extent of disease and type of surgery; n=89) with pancolitis and no reported BWI were also obtained from this database. In each case, all sections of the terminal ileum (TI) were reviewed, including sections from the ileocecal valve. The following histologic features were recorded: active inflammation, scored 0-4+ (0: no active inflammation; 1+: cryptitis; 2+: scattered crypt abscesses; 3+: numerous crypt abscesses; 4+: ulceration), presence or absence of features of chronicity and distribution of injury (none, focal/patchy, diffuse). In addition, all

sections of the cecum were reviewed to confirm the presence of pancolitis in study and control cases. If cecal involvement could not be documented, the case was excluded from further analysis (n=12). Histologic features were correlated with a number of short- and long-term complications with a mean follow-up of 5.7 yrs.

**Results:** After review of histologic sections, we identified 89 pts with some abnormality of the TI with cecal involvement and 80 pts with a normal TI and cecal involvement. Of these 169 pts, 135 had at least 1 complication, including pouchitis (n=55; 40.7%), wound infection (n=14; 10.3%), pelvic sepsis (n=19; 14.1%), pouch failure (n=8; 5.9%), bowel obstruction (n=33; 24.4%), hemorrhage (n=8; 5.9%), pouch fistula (n=18; 13.3%), anastomotic stricture (n=33; 24.4%), or anastomotic separation (n=15; 11.1%). Active inflammation of the TI was associated with anastomotic separation (p=0.0059) and bowel obstruction (p=0.04). No other histologic feature was associated with any of the other short- or long-term complications.

**Conclusions:** Close examination of sections of the ileocecal valve and TI in TPC specimens for UC is of clinical significance, since the presence of active inflammation is predictive of anastomotic separation and bowel obstruction.

### 438 Epidermal Growth Factor Receptor (EGFR) Protein, p53, and Cyclin D1 Expression in Colon Carcinoma: Correlation with EGFR Gene Amplification Using a Chromogenic In Situ Hybridization (CISH) Assay

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**Background:** Epidermal growth factor receptor (EGFR) is reportedly overexpressed in colon carcinoma. With the availability of anti-EGFR antibodies the accurate assessment of EGFR status has become an important test in many laboratories. Paraffin immunohistochemistry is the preferred method for assessing EGFR expression. Correlation between EGFR gene amplification and protein overexpression has not been thoroughly investigated in colon carcinoma and it is uncertain whether gene amplification is the underlying mechanism of EGFR protein upregulation. In this study 28 cases of colon cancer were analyzed for expression of EGFR, p53, and Cyclin D1 by paraffin immunohistochemistry. EGFR gene amplification was assessed using a chromogenic in situ hybridization (CISH) assay.

**Design:** A tissue microarray block was constructed using 28 cases of previously diagnosed colon adenocarcinoma arising within adjacent adenomatous epithelium. Two 0.6 mm cores were obtained from both the invasive tumor and adjacent adenomatous epithelium for a total of four cores per donor block. Four cores of both placenta and normal colon epithelium were utilized as controls. Paraffin immunohistochemistry was accomplished using a heat induced epitope retrieval followed by incubation with EGFR, cyclin D1, and p53 antibodies. Gene amplification was detected by a CISH method using probes and reagent kit as recommended by the manufacturer. EGFR gene amplification was analyzed using light microscopy.

**Results:** In 8/28 cases multiple copies of the EGFR gene (average 4-5 signals) were identified indicating gene amplification. In the remaining 20 cases only 2 intranuclear dot-like peroxidase positive signals were present consistent with non-amplified gene. EGFR protein overexpression was identified in 7/28 cases including 3 cases with EGFR gene amplification. Cyclin D1 protein was expressed in 16/28 cases and p53 in 9/28 cases respectively. In four tumors both p53 and Cyclin D1 were expressed.

**Conclusions:** Our study reveals a poor correlation between EGFR gene amplification and protein overexpression. These findings may indicate that other mechanisms besides gene amplification are involved in EGFR protein deregulation. Therefore gene amplification assays may not be suitable for selection of patients who may benefit from anti-EGFR therapy. Cyclin D1 and p53 appear to be independent prognostic markers without significant correlation with EGFR upregulation.

### 439 Detection of Apoptosis in Colorectal Tissues Using M30 Immunohistochemistry

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**Background:** Current techniques employed to detect apoptosis are recognised as difficult to interpret and have sub-optimal specificity. A neo-epitope in cytokeratin 18, the M30 antigen, becomes available early in the caspase cleavage stage of apoptosis. M30 antigen is detected using a monoclonal antibody in cells of many types of epithelial tissue and is not present in necrotic or vital cells. Recent studies have supported the use of M30 to detect apoptosis in colorectal mucosa. The aim of this study was to assess the use of M30 in colorectal tissue to detect the expected variation in apoptosis between normal mucosa, mucosa adjacent to colorectal adenocarcinoma, and colorectal adenocarcinoma.

**Design:** Paraffin-embedded surgical resection specimens from patients with and without colorectal neoplasia were immunostained for M30 antigen. Apoptotic indices (AI) were calculated by counting the proportion of mucosal cells with positive staining.

**Results:**

Table 1

| | Normal mucosa (n=17) | Mucosa 10cm from adenocarcinoma (n=33) | Adenocarcinomas (n=35) |
|---|---|---|---|
| Median AI% (IQR) | 0.00 (0.00-1.26) | 0.00 (0.00-0.55) | 0.00 (0.00-0.13) |
| Mean AI% (sd) | 1.06 (1.99) | 1.58 (3.16) | 0.25 (0.94) |
| No of M30 positive specimens (%) | 5 (29%) | 8 (24%) | 9 (26%) |

IQR interquartile range sd standard deviation

AI was not normally distributed, and the prevalence of M30 immunostaining was less than 50% in all groups. Although mean AI's showed some differences, there were no significant differences using non-parametric (Mann-Whitney) testing.

**Conclusions:** M30 immunohistochemistry detects less than expected apoptosis in colonic epithelium, which is recognised as a high turnover epithelium. Although altered apoptosis is thought to be important in the development of carcinoma, M30 immunohistochemistry does not significantly detect such alteration on non-parametric testing. These results question studies to date, which rely on mean M30 values and parametric tests of difference. Further attention to M30 sensitivity may be required before it can be usefully employed as a marker of apoptosis in colorectal tissues.

### 440 Microsatellite Instability Does Not Correlate with Clinicopathologic Features in Gastric Cancers

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**Background:** Microsatellites instability(MSI) is an indicator of deficient mismatch repair that is an important pathologic mechanism of hereditary nonpolyposis colorectal cancer (HNPCC). The stomach is a frequent site of extracolonic cancer development in patients with HNPCC and is one of the organs in which primary sporadic tumors show MSI+ phenotype. We analyzed the MSI data of 221 Korean gastric cancers to know the incidence and clinicopatholgic features of MSI+ gastric cancers.

**Design:** Microsatellites analysis was performed by PCR using 5 markers (BAT25, BAT26, D5S346, D2S123, and D17S250) and the results were classified into MSI-stable, MSI-low, and MSI-high according to the recommendations by National Cancer Institute. The expressions of hMLH1 and hMSH2 protein were evaluated immunohistochemically.

**Results:** Twenty patients (9.1%) had MSI+ gastric cancers. One showed MSI-low and 19 patients showed MSI-high. There was no difference in gender, age, tumor location (upper *vs* middle *vs* low), histology (tubular adenocarcinoma *vs* non-tubular adenocarcinoma), tumor invasion (EGC *vs* AGC), and multiplicity between MSI+ and MSI-stable tumors. Loss of hMLH1 expression was found in 13/17 (76.5%) of MSI+ and 5/134 (3.7%) of MSI-stable tumors ($p<0.001$). hMSH2 expression was markedly decreased in 1/17 (5.9%) of MSI+ and 2/133 (1.5%) of MSI-stable tumors (p=0.225). Seven (35%) MSI+ patients had one or more family members with gastric cancer or other malignancies (leukemia, carcinoma of colon and lung).

**Conclusions:** We detected MSI+ phenotype in 9.1% gastric cancers. Loss of hMLH1 expression could be a powerful predictor of MSI status. We failed to detect clinicopathologic features to distinguish MSI+ phenotype from MSI-stable gastric cancers. Although MSI+ phenotype is suggestive of genetic susceptibility for cancer development, we think that mutation analysis for hMLH1 and hMSH2 should be followed by MSI analysis in MSI+ patients to discriminate familial MSI+ from sporadic MSI+ gastric cancers.

### 441 Loss of CDX2 Expression Correlates with Clinicopathologic Features in Colorectal Cancers

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**Background:** The caudal-related homeobox transcription factor, CDX2, plays an important role in proliferation and differentiation of intestinal epithelial cells. Its expression is restricted to normal and neoplastic intestinal epithelium from duodenum to rectum, intestinal metaplasia of the stomach, and intestinal-type gastric cancer. We evaluated CDX2 expression in advanced colorectal cancers to determine the correlation between CDX2 expression and clinicopathologic features.

**Design:** 420 consecutive colorectal cancers were included in this study. CDX2 expression was investigated by immunohistochemistry using tissue microarrays constructed from surgically resected specimens. 148 invasive breast cancers, normal gastric mucosa, liver, endometrium, lung, kidney, and ovary were used as control. Only nuclear staining was considered as positive and the result was divided into 3 categories with the followings: <10% of cells staining = negative, 10-50% of cells staining = weakly positive, and >50% of cells staining = strongly positive.

**Results:** In 380 of 420 (90.5%) cases, CDX2 was expressed. 349 of 380 (83.1%) cases showed strong and diffuse immunostaining and 31 of 420 (7.4%) cases showed weakly positive staining. Forty patients (9.5%) were negative for CDX2. All of the invasive breast cancers, as well as all non-neoplastic control tissues, were negative for CDX2, however regions of intestinal metaplasia in gastric mucosa showed strong CDX2 expression. Loss of CDX2 expression was observed in cases with deeper invasion (p=0.03), lymph node metastasis (p=0.001), poor histologic differentiation ($p<0.001$), and distant metastasis (p=0.01).

**Conclusions:** CDX2 is a highly sensitive marker to detect intestinal origin metastases and might be a novel prognostic marker in advanced colorectal cancers.

### 442 Glucose Transporter 1 (GLUT-1) Expression and Cellular Localization in Early Colorectal Neoplasia

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**Background:** Glucose Transporter 1(GLUT-1) is a high-affinity glucose transporter. Increased glucose uptake and utilization is one of the metabolic characteristics of tumour cells. Elevated levels of GLUT-1 have been reported in many human malignancies, however the significance of GLUT-1 localization and expression in colorectal cancer (CRC) and corresponding adenomas has not yet been elucidated.

**Design:** The object of this study was to investigate the expression and localization of GLUT-1 in CRC specimens and adenomas using tissue microarrays and immunohistochemistry. To date, we have analysed 106 CRC cases (Dukes A & B) and 21 adenomas (11 contiguous to carcinoma and 10 separate). Four 6mm cores from each sample were scored and assessed for percent positivity and cellular localisation. Results were correlated with clinicopathological features.

**Results:** 96/106 CRC cases (91%) showed positive GLUT-1 immunostaining and 10 cases (9%) were negative. Three patterns of staining were observed; supranuclear, membranous and mixed. GLUT-1 expression in CRC showed supranuclear staining in 37 cases (38%), membranous in 44 cases (46%) and mixed in 15 cases (16%). In the adenomas examined, 14 displayed supranuclear staining, 3 membranous and 4 were negative. The 3 cases with membranous staining were contiguous adenomas, 2 of which had high-grade dysplasia. The carcinomas, which developed in these patients, showed either a membranous or mixed pattern. Comparison of GLUT-1 expression and clinicopathological characteristics in all cases revealed significant associations between high GLUT-1 expression and Dukes stage B ($p < 0.001$), high T stage ($p<0.001$), level of invasion ($p=0.005$) and peritoneal involvement ($p=0.006$).
**Conclusions:** Our findings indicate that both the increased expression and cellular localization of GLUT-1 may be useful determinants of neoplastic progression in CRC. We hypothesise that translocalisation of GLUT-1 from a supranuclear to a mixed or membranous pattern may correlate with CRC progression.

### 443 Prediction of Celiac Disease with Transglutaminase Autoantibodies Measured by Radioassay Versus Elisa Assay

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**Background:** It is now possible to identify children at risk for celiac disease and to identify those with "celiac autoimmunity" based on seropositivity for transglutaminase autoantibodies (TGAA). We have previously shown that the autoimmune process can lead to widely fluctuating levels of TGAA that correlate with variable findings on small bowel biopsy. In our assay, setting a higher TGAA level threshold before undergoing biopsy from 0.05 (the cutoff for a positive value) to 0.5 may minimize risk of endoscopy in healthy asymptomatic children. Because of these fluctuations, TGAA levels obtained at the time of biopsy may be a better predictor of intestinal pathology. However, this is true for the radioassay (RIA) to a much greater extent than for the ELISA assay. We questioned whether TGAA measured by RIA is superior to ELISA in quantitative assessment to predict celiac disease.
**Design:** Sera from 10 healthy controls and 31 genetically-risk children identified on screening with a RIA as expressing TGAA and on a normal diet were evaluated using 2 TGAA assays: The QUANTA Lite h-tTG kit from INOVA using human red blood cell TG substrate-based ELISA, and the in-house human recombinant 35S-labeled TG RIA. Intestinal biopsies were obtained at the time of sampling, Marsh 2 or 3 was considered positive for celiac disease, Marsh 1 was indeterminate (these were excluded), Marsh 0 was negative. Both IgA and IgG were measured in all samples for each assay format.
**Results:** For TG IgA, the RIA and ELISA systems correlated well ($r = 0.79$). The ELISA was unable to further quantify TGAA levels beyond an index of 120. For TG IgG, there was less correlation between the two assays for TG IgG. TG IgA correlated with TG IgG in the RIA system ($r=0.88$) better than in the ELISA system ($r=0.55$). The ELISA failed to detect TG IgG autoantibodies in majority of the patients with celiac disease confirmed by intestinal biopsy (1/18).
**Conclusions:** The RIA detection system is superior to the ELISA in assessing higher IgA TGAA levels. The ELISA was unable to further quantify IgA TGAA levels beyond an index of 120, and it failed to detect IgG TGAA because of saturation of the TG antigen by the large amount of IgA TGAA in the patient's serum. A higher TGAA index in either the RIA or ELISA assay may be used for selecting screening-identified individuals for biopsy to confirm CD.

### 444 *BRAF* Mutations in Hyperplastic Polyposis

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**Background:** Patients with hyperplastic polyposis have multiple hyperplastic polyps (HPs) and increased incidence of colorectal carcinomas. *BRAF* is an oncogene, and mutations of *BRAF* gene have been reported in colorectal cancers, serrated adenomas and hyperplastic polyps. However, the role of *BRAF* mutations in patients with hyperplastic polyposis remains unclear.
**Design:** We evaluated *BRAF* mutations by DNA sequencing in 70 HPs, 7 serrated adenomas, 10 tubular adenomas, and 6 carcinomas from 17 patients with hyperplastic polyposis. *BRAF* mutation status was associated with the clinicopathologic features and other genetic alterations by marginal logistic regression.
**Results:** *BRAF* mutations were present in 43% (30 of 70) of HPs, 57% (4 of 7) of serrated adenomas, 30% (3 of 10) of tubular adenomas, and 33% (2 of 6) of carcinomas from patients with hyperplastic polyposis. *BRAF* mutation status was concordant within HPs from the same patient (odds ratio 2.9, $p=0.02$), and were associated with younger age (odds ratio 0.9, $p=0.002$), patients with a large HP (odds ratio 18.4, $p=0.01$), HPs from the right colon (odds ratio 2.3, $p=0.001$) and methylation of MINT31 locus (odds ratio 4.5, $p=0.0005$).
**Conclusions:** *BRAF* mutations are common in hyperplastic polyps and other lesions from patients with hyperplastic polyposis and may have an important role in the pathogenesis of colorectal neoplasia in these patients.

### 445 CD117 (c-Kit) in Anorectal Melanoma-A Potential Diagnostic Pitfall

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**Background:** Following 2 small biopsies of CD117 positive rectal spindle cell tumors which proved to be anorectal malignant melanoma (ARMM), we stained a series of ARMM for CD117. Our purpose was to determine the prevalence of CD117 in ARMM and its possible relationship with prognosis and other clinical and pathologic parameters.
**Design:** Thirty cases of ARMM were stained for CD117 (polyclonal, DAKO, 1:400) following HIER.CD117 stain was considered positive when more than 10% cells stained. All cases were positive for at least 2 of 3 melanoma markers (S100, HMB45, Melan A). Clinical data (age, surgical procedure, survival, cause of death) and pathologic data (tumor size, tumor depth, mitotic activity, vascular invasion, Ki67 index) were also evaluated. Univariate and multivariate survival analysis were performed.
**Results:** Positive CD117 stain, usually both membranous and cytoplasmic, was detected in 18 of the 30 cases, It was strong and diffuse (over 50% of cells) in 11 cases, weak to moderate and focal (less than 50% of cells) in 7 cases. It was not associated with the clinical or the other pathologic parameters.
Survival ranged from 1 to 140 months (for 27 patients, 2 are recent cases, 1 with no follow-up), with only 3 long-term survivors (140, 120 and 96 months) and 22 who died of disease. 12 patients underwent local excision (LE), 11 abdominoperineal resection (APR), 5 were not operated. Complete tumor excision (by LE or APR) with no distant metastases at diagnosis was achieved in only 17 cases. Tumor size ranged from 1.5 to 11 cm, tumor depth from 6 to 40 mm. Mitotic rate>20 per 10 high-power fields was seen in 12 cases. Vascular invasion was detected in 3 cases. Ki67 index ranged from 29% to 64%.
By univariate analysis decreased survival was associated with tumor depth>10 mm ($p=0.036$), tumor size>3 cm ($p=0.029$) and Ki67 index>33% ($p=0.014$), but not with CD117 stain, vascular invasion, higher mitotic rate or age. By multivariate analysis only higher Ki67 index ($p=0.036$) was associated with reduced survival.
**Conclusions:** 1. CD117 is frequently detected in ARMM. To prevent misdiagnosis of rectal GIST, additional immunohistochemical stains, including melanoma markes should be performed.
2. CD117 was not associated with prognosis in this series of ARMM. However, due to the limited number of patients treated with attempt to cure (complete tumor excision and no distant metastases at diagnosis), and the very small number of long-term survivors, larger series should be studied.
3. Treatment with tyrosine-kinase inhibitors (STI571) could be considered in ARMM.

### 446 Overexpression of the High Mobility Group Proteins HMGI(Y) in Dysplasia Arising in Ulcerative Colitis

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**Background:** Determining whether a dysplastic lesion in a colon biopsy from a patient affected by ulcerative colitis is a DALM or sporadic adenoma (SA) can be difficult on morphologic grounds. The aim of this study is to determine whether immunostaining for the high mobility group proteins HMGI(Y) can distinguish between these two lesions.
**Design:** Sections from 11 DALMS from 11 different colectomies and 22 endoscopically removed adenomas from patients with no history of IBD were stained for HMGI(Y) using the immunoperoxisae method. The percentage of positive cells was scored on a scale of 0-5 with 0 completely negative; 1, 1-10%; 2, 11-25%; 3, 26-50%; 4, 51-75%, and 5, >75% of the dysplastic cells positive for HMGI(Y). The intensity of staining was scored as 0, completely negative; 1, weak; 2, moderate; and 3, strong nuclear staining. The HMGI(Y) immunostaining score was determined by multiplying the percent positive score by the intensity score.
**Results:** HMGI(Y) immunoreactivity was always nuclear. Whereas 10 (91%) of the 11 DALMS had a score >4, only 4 (18%) of the SA had a score >4 ($p = 0.0001$, Fisher's Exact Test, Relative Risk 13.57, 95% Confidence Interval: 1.957-94.131).
**Conclusions:** Our results strongly indicate that HMGI(Y) is significantly overexpressed in DALM compared to SA, and suggest that immunostaining for HMGI(Y) may help distinguish DALM from SA.

### 447 Molecular Detection of *Campylobacter jejuni* in Archival Cases of Acute Appendicitis

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**Background:** The role of enteric bacteria in the pathogenesis of acute appendicitis (AA) has been a subject of great debate. Studies using microbiologic culture techniques have isolated a wide variety of bacteria from acutely inflamed appendices, including *Campylobacter jejuni* (CJ), the most common stool pathogen in the United States. CJ has been demonstrated in a minority of cases of AA using microbiological and immunohistochemical techniques, notably in cases where inflammation was limited to the mucosa/submucosa (similar to many cases of bacterial colitis), without transmural suppuration or mural necrosis. Our goal was to evaluate cases of AA for CJ DNA using molecular methods.
**Design:** Fifty cases of AA were reviewed, with attention to numerous histologic features including mucosal ulceration, cryptitis, depth of inflammatory infiltrate, and presence of mural necrosis. PCR was performed from archival tissue using primers designed to target a 287-bp fragment of the mapA gene that is specific to CJ. Beta-actin served as a housekeeping gene to ensure intact DNA, and pure CJ DNA derived from cultures served as the positive control. Twenty histologically unremarkable incidental appendectomy specimens served as negative controls.
**Results:** Twenty-two percent (11/50) of AA cases were positive for CJ DNA by PCR analysis. Control cases were all negative for CJ DNA. All patients presented with signs and symptoms typical of AA. Overall, the histological features of CJ-positive cases were indistinguishable from CJ-negative cases. However, 3/11 CJ-positive (27%) cases contained acute inflammation limited to the mucosa/submucosa rather than transmural inflammatory infiltrates, 6 (55%) lacked mural necrosis, and 3 (27%) lacked both mural inflammation and necrosis.
**Conclusions:** *C. jejuni* DNA was detected in a significant percentage (22%) of AA cases. CJ is an enteric pathogen that does not exist as a commensal or nonpathogenic organism, thus the presence of CJ DNA implies current infection. Similar to previous

microbiological and immunohistochemical studies, CJ-positive AA cases often have inflammation limited to the mucosa/submucosa, and lack mural necrosis. Further study is needed, but the presence of CJ DNA in an inflamed appendix (particularly one lacking transmural inflammation and necrosis) may indicate appendiceal involvement by CJ enteritis, rather than a true causative role for CJ in acute appendicitis.

### 448 Cell Cycle Regulators and Apoptosis-Related Proteins in Epstein-Barr Virus Associated Gastric Carcinomas

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**Background:** Few studies have been done on overview of gene expression profiling in Epstein-Barr virus associated gastric carcinomas.

**Design:** Fifty-five cases of Epstein-Barr virus (EBV) (+) gastric carcinomas were established using in situ hybridization for EBV-encoded small RNAs and tissue array technique. The expression of 20 proteins (cell cycle regulators and apoptosis-related proteins) and the prognostic value of these markers were evaluated. RT-PCR and Western blot were performed in EBV(+) stomach cancer cell line.

**Results:** In immunohistochemistry, bcl-2 positive (in 2% of EBV(+) carcinomas), p53 overexpression (in 7%) and Rb loss (in 4%) were rare. Meanwhile, p27 (loss in 85%), p16 (loss in 78%), cyclin D1 (overexpression in 65%), and positive for TRAIL (67%), BAX (64%), caspase-1 (62%), and survivin (75%) were frequent. The tumor size, TNM stage, p16, Fas, FADD, and NFκB were related to overall survival ($p < 0.05$), but TNM stage was the only independent prognostic factor. Among 11 stomach cancer cell lines, EBV(+) and BARF1 mRNA (+) by RT-PCR were in a cell line, SNU 719, in which bcl-2 using Western blot was expressed less than those in EBV(-) stomach cancer or EBV(+) lymphoma cell lines.

**Conclusions:** The bcl-2 activation and p53-Rb pathway rarely contribute to the development in EBV(+) gastric carcinomas, while p27, p16, cyclin D1, TRAIL, BAX, caspase-1 and survivin may be important.

### 449 Evaluation of Activated Nuclear Factor KB (NF-κb) in Gastric Adenocarcinoma

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**Background:** The incidence of gastric adenocarcinoma is markedly increased in patients with chronic gastritis associated with intestinal metaplasia. However, the mechanism of tumor development remains poorly understood. Our in vitro studies revealed that intestinal trefoil factor (TFF3) activate NF-κb and up-regulate cyclooxygenase-2 (COX-2) expression in rat intestinal epithelial cells, resulting in increased resistance to apoptosis. We also demonstrated that a majority of intestinal type of gastric adenocarcinomas co-overexpress TFF3 and COX-2. Based on these findings, we hypothesize that NF-κb plays an important role in the antiapoptotic effect of TFF3 by induction of COX-2 expression, thus contributing to gastric carcinogenesis. The objective of this study is to evaluate the activation of NF-κb and correlate with the expression of TFF3 and COX-2 in gastric adenocarcinoma.

**Design:** Formalin-fixed and paraffin-embedded tissue sections from 47 gastrectomy specimens resected for adenocarcinoma were used in this study. The ABC-immunoperoxidase method was employed to evaluate the expressions of activated NF-κb (monoclonal antibodies against NF-κb p65 subunit), TFF3 (polyclonal antibodies) and COX-2 (monoclonal antibodies). Immunostains were evaluated for intensity and number of cells stained.

**Results:** In normal gastric mucosa, the staining of NF-κb was negative in the foveolar epithelium and positive in fundic and pyloric glands. Activated NF-κb was observed in 62% adenocarcinomas. Increased expression of TFF3 and COX-2 was observed in 70% and 89% adenocarcinomas respectively. Activation of NF-κb was seen in 76% of cases with elevated expression of TFF3. Among NF-κb positive cases 86% of the tumors overexpressed both TFF3 and COX-2 proteins. Interestingly, 15 tumors (32%) with overexpression of COX-2 did not show activation of NF-κb, indicating that in addition to NF-κb other factors may also involved in the up-regulation of COX-2.

**Conclusions:** The results of this study demonstrate that activation of NF-κb is frequent in intestinal type of gastric adenocarcinomas, and is associated with overexpression of TFF3 and COX-2. These findings support our hypothesis that NF-κb is an important mediator in the antiapoptotic pathway of TFF3 and contribute to the pathogenesis of gastric carcinomas.

### 450 Differential Expression of α-Methylacyl Coenzyme A Racemase in Adenocarcinomas of the Small and Large Intestines

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**Background:** α-Methylacyl coenzyme A racemase (AMACR) serves an essential role in β-oxidation of branched chain fatty acids and bile acid intermediates. It is a newly identified biomarker for prostate cancer but its overexpression has also been reported in a number of malignancies including colorectal adenocarcinoma. However, AMACR expression has not been investigated in primary adenocarcinoma of the small intestine, an uncommon malignancy with morphologic features similar to, or indistinguishable from, colorectal adenocarcinoma.

**Design:** Fifty-nine surgically resected primary small intestinal adenocarcinomas (34 ampullary and 25 non-ampullary) were included in the study. Formalin-fixed paraffin-embedded tissue blocks were selected to include both normal-appearing intestinal mucosa and tumors. Immunohistochemical staining for AMACR was performed using a prediluted polyclonal antibody (Biocare Medical). A case was considered positive if more than 5% of the tumor cells exhibited cytoplasmic staining. For comparison, 73 colorectal adenocarcinomas (including 23 tumors secondarily involving the small intestine) were similarly examined.

**Results:** No AMACR immunoreactivity was detected in normal-appearing small and large intestinal mucosa. While 46 of 73 (63%) colorectal adenocarcinomas exhibited a strong cytoplasmic staining pattern, only 1 of 25 (4%) non-ampullary and 2 of 34 (6%) ampullary small intestinal adenocarcinomas showed positive AMACR immunoreactivity ($P<0.0001$). Interestingly, among colorectal adenocarcinomas, only 1 of 9 (11%) mucinous carcinomas stained positive for AMACR. This was statistically different from non-mucinous variants where AMACR expression was observed in 45 of 64 (70%) cases ($P=0.011$).

**Conclusions:** AMACR is frequently overexpressed in colorectal adenocarcinomas, particularly those with a non-mucinous morphology. More importantly, our findings that AMACR overexpression is an infrequent event in small intestinal adenocarcinomas may not only provide a useful diagnostic tool in aiding the distinction between adenocarcinomas of the small and large intestinal origins, but also shed light on further understanding of intestinal tumorigenesis.

### 451 Colorectal Carcinomas Lacking MGMT Immunohistochemical Expression Display a Peculiar Clinicopathologic Profile

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**Background:** $O^6$-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that removes mutagenic and cytotoxic alkyl adducts from $O^6$-guanine in DNA. It is transcriptionally silenced by promoter hypermethylation in several human cancers, including colorectal carcinomas (CRCs). Little is known about clinicopathologic features of MGMT methylated CRCs.We analyzed MGMT expression and methylation status in 44 non selected CRCs.

**Design:** Formalin-fixed, paraffin-embedded tissue sections were dewaxed, microwave treated and immunostained with a mouse anti-MGMT antibody (clone MT3.1; Chemicon Int., CA). Only nuclear immunoreactivity (IR) has been considered; normal epithelium and stromal cells provided a positive internal control. A tumor with IR in less than 10% of cells was considered MGMT negative (MGMT-). The methylation status of MGMT promoter was analyzed by DNA modification with sodium bisulfite and following Methylation-Specific PCR using primer sets specific for methylated and unmethylated sequences.

**Results:** Twelve of the 44 cases (27%) did not show any IR for MGMT. Promoter methylation was observed in all these cases (100%) and in 1/6 (17%) MGMT-positive (MGMT+) CRCs (p=0.001). MGMT- CRCs were more frequent in females than in males (M/F: 0.5 in MGMT- versus 1.2 in MGMT+ CRCs).

A significant association was found between MGMT- CRCs and histological aspect: all the MGMT- cases were moderately (G2) differentiated CRCs and 7/12 (58%)displayed complete (4 cases) or consistent (10-40% of tumor, 3 cases) mucinous differentiations. Mucinous areas were observed in only 3/32 (9%) MGMT+ CRCs (p=0.004). Absence of MGMT-IR was also positively correlated with the presence of a polypoid-fungating macroscopic appearance, observed in 7/8 (87.5%) MGMT- versus 6/23 (26%) MGMT+ CRCs (p=0.009) and with the right colon location, found in 7/12 (58%) MGMT- versus 10/32 (31%) MGMT+ CRCs.

Lymph node metastases were observed in 2/12 (17%) MGMT- versus19/29 (65.5%) MGMT+ cases (p=0.01).

No relationship was observed between MGMT expression and high level microsatellite instability (MSI) or p53 overexpression.

**Conclusions:** Immunohistochemical expression of MGMT is a good method to identify the presence of MGMT promoter methylation. Transcriptional silencing of MGMT gene seems to be involved in a subset of CRCs, distinct from MSI tumors. They occur more frequently in females, in the right colon and are characterized by a polypoid growth, mucinous differentiation and low rate of lymph node metastases.

### 452 Site-Specific Ectopic Expression of Gastric Pyloric Mucin MUC6 in Colonic Hyperplastic Polyps and Serrated Adenomas

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**Background:** Colorectal carcinoma with microsatellite instability is commonly found in the proximal colon of women. Recent studies identified its precursors as hyperplastic polyps (HPs) and serrated adenomas (SAs). The mechanism of its site and gender predisposition is still unknown. Gastric differentiation has been shown in both HPs and SAs. MUC6 is a gastric mucin expressed in the deep pyloric glands. pS2 is a trefoil factor (TFF1) that is expressed in the surface foveolar epithelium of the stomach but not in the colon. Both MUC6 and pS2 have been shown to be estrogen- and steroid-inducible in breast cancer cell lines. The aim of this study is to compare the expression of MUC6 and pS2 in proximal and distal colonic HPs and SAs.

**Design:** Biopsies of 40 HPs, 18 SAs, and 20 tubular adenomas (TAs) from the proximal and distal colon were retrieved from the surgical pathology file. Formalin-fixed paraffin-embedded blocks were sectioned and stained with monoclonal antibodies to MUC6 and pS2 using standard methods.

**Results:** Antibody to pS2 stains most HPs and SAs but not tubular adenomas. pS2 is diffusely present in the mucinous granules of the upper three-quarters of the hyperplastic mucosa in HPs and SAs. There is no significant difference in the extent and intensity of staining between the proximal and distal HPs and SAs. In contrast, the immunostaining of MUC6 is limited to the lower crypt cells of hyperplastic mucosa. Its expression is independent of patient's age, sex, or histological type (HP vs. SA). The difference of MUC6 expression is significant between proximal and distal HPs ($p<0.000001$) and SAs ($p<0.0001$). In 2 cases of SAs with high-grade dysplasia, MUC6 expression extended to the entire crypts that were involved by high-grade dysplasia which also showed loss of MLH1 mismatch repair protein.

| | Proximal HP (N=20) | Distal HP (N=20) | Proximal SA (N=8) | Distal SA (N=10) | TA (N=20) |
|---|---|---|---|---|---|
| MUC6(+) | 18/20 | 2/20 | 7/8 | 1/10 | 1/20,focal |
| pS2(+) | 19/20 | 16/20 | 7/8 | 7/10 | 0/20 |

**Conclusions:** The similar distribution of pS2 and MUC6 in colonic hyperplastic polyps and serrated adenomas suggest that both types of polyps are variants of the same lineage. The site-specific expression of MUC6 in the colon supports the theory that proximal colon differs from the distal colon in molecular regulation of antigen expression and possibly different response to hormonal stimuli such as estrogen. Ectopic expression of MUC6 may be an early marker for the risk of malignant transformation in colonic hyperplastic polyps and serrated adenomas.

### 453 Size and Number of Negative Lymph Nodes Impact Outcome in Patients with Node-Negative Stage II Colorectal Cancer

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**Background:** Published analyses concluded that pathologic assessment of negative lymph nodes in colorectal cancer surgical specimens is often suboptimal and that examining greater numbers of nodes increases the likelihood of proper staging of stage II tumors. The number of lymph nodes may reflect surgical technique and thoroughness of pathologic dissection. We postulated that size in addition to number of negative nodes could impact survival.

**Design:** We evaluated 150 consecutive patients with available pathologic material of node-negative adenocarcinoma of the colon and rectum at The University of Texas M. D. Anderson Cancer Center between 1990 and 1998. H&E sections were reviewed retrospectively without knowledge of patient follow-up status. All glass slides with lymph nodes were then digitally scanned and the surface area of the nodes recorded. Various clinicopathologic features, including number and size of lymph nodes (mean, median, sum, maximum), and demographic data were compared with outcome.

**Results:** The mean number of lymph nodes examined was 12.5 (95% confidence interval 10.9-14.0, range 0-51). As in previous reports, a larger number of examined lymph nodes was associated with better overall survival: median survival was 127 ± 5.75 (standard error) months for patients with more than eleven lymph nodes as contrasted with 105 ± 7.21 months (p=0.02). In addition, the median overall survival of patients with larger lymph nodes was longer: 127 ± 5.85 months vs. 105 ± 7.03 months (p=0.01). The sum of the sizes of the nodes was a significant predictor for survival (p=0.005). Overall survival was best in patients with large numbers of large nodes and worst in those with small numbers of small nodes. In multivariate analysis, overall survival was independently predicted by the size of negative lymph nodes (p=0.01).

**Conclusions:** The size as well as the number of negative lymph nodes examined in resection specimens significantly predict overall survival in patients with node-negative adenocarcinoma of the colorectum. We speculate that high numbers of large negative lymph nodes are due to host response to the cancer that contributes to improved survival.

### 454 Morphologic and Immunohistochemical Features of Anorectal Melanoma

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**Background:** Primary anal malignant melanoma is a rare, highly lethal neoplasm. Due to its rarity and tremendous histologic variability, misdiagnoses as lymphoma, carcinoma or sarcoma are common. Although the morphologic patterns have been reported in small series, the immunohistochemial staining of these lesions has not been previously described. We review the morphologic and immunohistochemical features of 17 anorectal melanomas.

**Design:** The University of Virginia Department of Pathology files were reviewed and a total of 20 cases, including both referral and in-house material, were identified from 1947 to 2004. Slides of seventeen cases were available for review and 12 had material, either paraffin blocks or unstained slides, for immunohistochemical evaluation. Morphologic features evaluated included junctional change, pigmentation, architecture, and mitotic rate. Immunohistochemical stains were performed for/with S-100, HMB45, MelanA, tyrosinase, vimentin, KIT and pankeratin. The staining was graded as follows: 0 no staining; 1+ <25% staining; 2+ 25-75% staining, 3+ >75% staining.

**Results:** The average age at presentation was 70.4 yrs (range 56-96) without a gender preference. Of 17 cases, epithelioid features were present in 13, spindle cell features in 10, lymphoma-like features in 9, and pleomorphic features in 3. Pigmentation was present in 12 cases, but was often sparse and easily missed. Junctional change was present in 12 cases. The mitotic rate was greater than or equal to 3 mitotic figures per high power field in 8 cases.

S100 staining was present in 12/12 cases (10/3+, 1/2+, 1/1+), HMB45 staining was present in 11/12 cases (8/3+, 2/2+, 1/1+), MelanA staining was present in 12/12 cases (7/3+, 2/2+, 3/1+), tyrosinase staining was present in 9/10 cases (9/3+), vimentin staining was present in 8/10 cases (5/3+, 2/2+, 1/1+), KIT staining was present in 12/12 cases (5/3+, 3/2+, 4/1+), and pankeratin staining was absent in all cases (0/11).

**Conclusions:** Anal malignant melanoma is a disease of the elderly that shows considerable histologic variability. Immunohistochemical staining is similar to that seen in cutaneous melanomas, with a high percentage positive for/with S100, HMB45 and MelanA, and uniform negativity for pankeratin. Tyrosinase shows more variability in expression. KIT expression was present in all cases, including spindle cell tumors, where it may lead to confusion with gastrointestinal stromal tumors (GISTs).

### 455 RACK1 Overexpression Associates with High Grade and Lymph Node Metastases in Colorectal Carcinomas: A Preliminary Study

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**Background:** The receptor for activated C kinase 1 (RACK1) is a protein kinase C-mediated (PKC) signaling enzyme. RACK1 is an adaptor protein that mediate protein-protein interactions, anchors activated PKC isozymes and other signaling enzymes and regulates cell growth. The function and levels of expression of RACK1 may help elucidate signaling pathways leading to carcinogenesis and could result in the identification of novel therapeutic targets.

**Design:** RACK1 expression was evaluated in 116 colorectal carcinomas between 2001 and 2003, to asses the clinical utility of the levels of expression of RACK1 protein. Tissue microarrays (TMAs) representative of normal mucosa and colorectal carcinoma of all cases were performed, including areas from the centre and infiltrative margin of the tumor. When present, additional TMAs from metastatic lymph nodes or adenomas where done. RACK1 levels were determined by immunohistochemical staining. Positivity was scored using the Hscore (range from 0 to 300) resulting from multiplying the intensity (0 to 3) by the percentage of positive staining. In a subset of tumors Western-blot analysis was also performed. Results were correlated with clinical, histopathological parameters, and survival.

**Results:** RACK1 overexpression was observed in 61'2% (71) cases. Overexpression of RACK1 correlated with poor differentiation (grade) p=0.036 and presence of lymph node metastases (p=0.049). No differences were observed between RACK1 expression and tumor localisation, stage or depth of tumor infiltration. Interestingly RACK1 expression in adenomas showed only 16'6% overexpression (2/12), compared to 70% in concomitant carcinomas (p=0.010). No differences in RACK1 immunostaining were observed between the centre of the tumor and the infiltrative margin, neither between the primary tumor and metastatic lymph nodes (p=0.927). Western-blot analysis confirmed these results. Disease-free survival was not related to RACK1 expression (p=0.356).

**Conclusions:** We have found significant differences in expression of RACK1 in high grade and lymph node metastatic colorectal carcinomas, related to adenomas and low grade carcinomas.These findings suggest that RACK1 may contribute to tumor progression. We propose RACK1 expression as a potential marker of worse prognosis in colorectal carcinomas. Further studies should be done in order to confirm if it could be used as a novel therapeutical marker.

### 456 Eosinophils in the Esophagus: Reflux or Eosinophilic Esophagitis?

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**Background:** Eosinophilic esophagitis (EE) is an increasingly recognized disease entity previously typified by dysphagia in young men with atopy. Earlier reports on EE have defined histologic criteria on the basis of an absolute number of eosinophils per microscopic high power field. Given the overlap of histologic features between reflux (GERD) and EE and the small numbers of cases reported, pathologists have been hesitant to adopt these criteria. We examine the validity of such criteria in a significantly larger cohort.

**Design:** Computerized records of two institutions were searched to identify all possible cases of EE during the previous 3.5 years. Control cases of GERD were used from the same time period. Approval was granted by the IRB and QA/ORB of the two institutions, respectively. Slides were reviewed by 2 GI pathologists, blinded to history and diagnosis. The number and distribution of intraepithelial eosinophils per hpf (5 fields); number of eosinophilic microabscesses (EM), basal cell thickness (BCT) and papillary length (PL) were recorded. Clinical history was obtained from the medical records or via additional reports from clinicians.

**Results:** Of 202 cases reviewed, 65 were confirmed as EE. 64 of 65 EE cases contained > 20 eos/hpf (avg. 51.6 ); 37 of 65 had a superficial distribution (SD) and 56 of 65 had at least 1 EM; BCT >33% and PL >70% were seen in 59 and 52 EE cases ($p < 0.0001$ for both). In the 137 GERD cases, 3 contained > 20 eos/hpf (range: 24.8 to 37.4), 10 displayed SD and 7 contained EM. BCT > 33% was seen in 20 and PL >70% in 64 GERD cases. Presentation with dysphagia, impaction and/or stricture was associated with EE ($p < 0.0001$ for each); while "reflux" and abdominal pain were associated with GERD ($p < 0.0026$ and 0.0033, respectively). EE patients were younger (avg. 32) than those with GERD (avg. 44.7) [$p < 0.0001$]. Gender predominance, seasonal variation, other presenting symptoms and histologic features were not statistically significant.

**Conclusions:** In our larger cohort of patients, intraepithelial eosinophils > 20/hpf, particularly in combination with eosinophilic microabscesses or superficial distribution were reliable indicators of EE especially in the context of dysphagia or impaction. Rarely GERD may have similar numbers of eosinophils, however, the vast majority have far fewer. We observed no seasonal variation and contrary to previous reports, only slight male predominance was found.

### 457 CDX2 Expression in Intestinal Metaplasia, Dysplasia, and Carcinoma of the Gallbladder: An Immunohistochemical Study

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**Background:** Intestinal metaplasia of gallbladder epithelium is commonly seen in association with gallbladder carcinoma and is thought to be part of a sequence of carcinogenesis from intestinal metaplasia to dysplasia, to carcinoma-in-situ, to invasive carcinoma. This proposed sequence has been based mainly on the histologic association of intestinal metaplasia with dysplasia and carcinoma of the gallbladder. CDX2 is a recently introduced immunohistochemical marker of an intestinal specific transcription factor that is key in the differentiation and maintainance of intestinal-type epithelium.

CDX2 has been shown to be present in tumors of intestinal origin and recently, in intestinal metaplasia associated with Barrett's esophagus. This study's aim was to investigate the expression of CDX2 in normal epithelium, intestinal metaplasia, dysplasia and carcinoma of the gallbladder to evaluate the role of this transcription factor in this proposed sequence of carcinogenesis.
**Design:** 13 cases of intestinal metaplasia, 19 cases of dysplasia (17 low grade dysplasia and 2 high grade dysplasia), and 3 cases of adenocarcinoma of the gallbladder were identified from the pathology database of Wilford Hall Medical Center. The formalin fixed, paraffin embedded tissue blocks from these cases were compared against 22 age matched control cases of gallbladder tissue removed for chronic cholecystitis and cholelithiasis. Routine H&E stained sections were evaluated in conjuction with standardized immunohistochemical staining for expression of the CDX2 antigen.
**Results:** We discovered very low staining of CDX2 in histologically normal epithelium of the gallbladder (only 1 of 21 control cases showed some weak nuclear staining with CDX2). Contrarily, there was a strong association of positive staining with histologically identified intestinal metaplasia (12 of 13 cases, or 92 percent, showed at least some staining with CDX2). Likewise, 68 percent of dysplasias (13 of 19 cases) showed positive staining with CDX2. Additionally, 2 of the 6 negative cases of dysplasia showed associated intestinal metaplasia that stained for CDX2. In the cancer cases, 66 percent (2 of 3 cases) showed positive staining with CDX2.
**Conclusions:** CDX2 is a reliable marker of intestinal metaplasia of the gallbladder. Additionally, a high level of immunohistochemical staining of dysplasia and carcinoma of gallbladder epithelium by CDX2 further supports the role of intestinal metaplasia as a precursor lesion in the sequence of carcinogenesis in the gallbladder.

### 458 The Mucin Core Polypeptide Expression Pattern of Fetal Cardiac-Type Mucosa Supports the Contention That Adult Cardiac Mucosa Has a Congenital Origin

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**Background:** It is currently debated whether cardiac mucosa (CM) is normally present or acquired by metaplasia in a setting of reflux. If CM is a native structure, it develops during gestation. Thus, autopsy specimens from the fetal gastroesophageal junction (GEJ) may be the ideal material to study this question. The mucin core polypeptide expression patterns of adult CM and Barrett's mucosa have been described previously. The aim of this study was to evaluate the immunostaining patterns of 5 mucin core polypeptides in the fetal GEJ and to compare these patterns with the adult situation.
**Design:** 49 autopsy specimens of the fetal esophagus and stomach [mean gestational age, GA: 22 w, range: 7-44 w] were step-sectioned and stained with hematoxylin and eosin to select sections showing the mucosal lining. The mucosae were classified in 7 types: foregut embryonic epithelium, multilayered ciliated and squamous epithelium, and cardiac-, oxyntocardiac-, fundic- and pyloric-type mucosa. Oxyntocardiac and cardiac mucosa were defined as simple columnar mucus-secreting epithelium with and without parietal cells, respectively. Selected sections were immunostained (ABC method) with monoclonal antibodies against MUC1, MUC2, MUC4, MUC5AC and MUC6 mucin core polypeptides. Staining was evaluated for location and compared between mucosal types.
**Results:** Foregut embryonic epithelium (7 w GA) stained for MUC1 and MUC5AC. Multilayered ciliated epithelium (14-30 w GA) and squamous epithelium (from 34 w GA) were positive for MUC4 in the superficial cells. Some mucus-secreting cells at the squamocolumnar junction stained for MUC1 and MUC5AC. The surface and pit cells of all simple columnar epithelia showed variable expression of MUC1 and MUC4, and constant strong expression of MUC5AC. MUC1 was also present in parietal cells. MUC6 was seen in mucous neck cells of fundic-type mucosa and in the deepest part of scattered glands in cardiac- and pyloric-type mucosa. No immunostaining for MUC2 was observed.
**Conclusions:** The mucin core polypeptide expression pattern of the fetal GEJ shared features with that described for the normal adult GEJ in all 49 cases examined. It differed from the pattern of Barrett's mucosa by the absolute absence of MUC2 expression. These findings support the conclusion that adult cardiac mucosa has an identifiable precursor in the fetus. This would then indicate that at least part of the adult cardiac mucosa has a congenital origin.

### 459 CDX-2 Is Specific for Intestinal-Type Differentiation: A Tissue Microarray Based Study of 629 Tumors from Different Sites

*LB De Lott, C Morrison, S Suster, DE Cohn, WL Frankel.* Ohio State University, Columbus, OH.
**Background:** CDX-2 is a nuclear transcription factor necessary for intestinal organogenesis, cell differentiation, and is normally expressed in intestinal epithelium. Expression of CDX-2 has been reported in intestinal metaplasia and intestinal adenocarcinomas. We studied CDX-2 expression in 629 tumors from various sites including; colon, liver, lung, pancreas, and endometrium to determine the specificity of CDX-2 expression for intestinal adenocarcinomas.
**Design:** Seventy-one cases of colorectal adenocarcinoma, 31 hepatocellular carcinomas, 69 neuroendocrine carcinomas of the lung (30 well/moderate and 39 poorly differentiated), 47 adenocarcinomas of the lung, 55 squamous cell carcinomas of the lung, 43 neuroendocrine carcinomas of the pancreas (well and moderately differentiated), 57 pancreatic adenocarcinomas, and 256 endometrial carcinomas were retrieved from archival files. Tissue cores from formalin-fixed, paraffin-embedded blocks were arrayed to make tissue microarrays of 0.6 mm, 1.5 mm, and 2.0 mm. Sections were stained with a monoclonal antibody against CDX-2 and evaluated by two pathologists. Five percent or greater staining in either core was interpreted as positive and controls stained appropriately.
**Results:** CDX-2 was positive in 51/71 (71.8%) colorectal cancers. All other tumor types showed only infrequent cases with staining for CDX-2. Of the positive tumors; 1/30 (3.3%) was a well/moderately differentiated neuroendocrine carcinoma of the lung, 2/43 (4.7%) were well/moderately differentiated neuroendocrine carcinomas of the pancreas, 1/47 (2.1%) was a moderately differentiated adenocarcinoma of the lung, 3/57 (5.3%, including 1 intraductal papillary mucinous carcinoma) were pancreatic adenocarcinomas, and 15/256 (5.9%, including 13 endometrioid, 1 serous, 1 adenosquamous) were endometrial carcinomas. No hepatocellular carcinomas, poorly differentiated neuroendocrine carcinomas of the lung, or squamous cell carcinomas of the lung showed staining for CDX-2.
**Conclusions:** CDX-2 was fairly specific for intestinal-type differentiation and was rarely seen in tumors from the other sites evaluated. Therefore, CDX-2 may be useful in determining the site of origin for metastatic tumors.

### 460 Differential Expression of Mucins (MUC1, MUC2, MUC4, MUC5AC and MUC6) in Microsatellite Stable and Unstable Colorectal Cancers and Correlation with CDX-2

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**Background:** Colorectal cancers (CRC) with microsatellite instability (MSI) have been reported to express MUC2 and MUC5AC to a greater degree than microsatellite stable (MSS) CRC. Mucinous CRC exhibit increased MUC2, MUC5AC, and MUC1 compared with non-mucinous tumors. We compared mucin expression in MSI and MSS CRC and investigated whether patient age, gender, tumor location and histology contributed to the differences. Mucin expression was then correlated with CDX-2, which has been suggested to control MUC2 gene expression *in-vitro*.
**Design:** Thirty-five MSI (all MSI-high) and 36 MSS (including 6 MSI-low) CRC were retrieved from archival files. Tissue microarrays were made from formalin-fixed, paraffin-embedded blocks (2 cores per block, 2 mm each). Tumors had been previously assessed for MSI using a modified Bethesda panel of microsatellite markers. Sections were stained with antibodies against MUC1, MUC2, MUC4, MUC5AC, MUC6 and CDX-2. Five percent or greater staining was interpreted as positive. Mucin expression was correlated with patient age and gender, MSI status, tumor location and grade/type, and CDX-2 expression.
**Results:** Differences between MSI and MSS CRC were significant for location (p=0.001), tumor type (p=0.016), and gender (p=0.044), but not age. MSI tumors were right-sided (29/35, 85.7%) with a more poorly differentiated tumors (15/35, 42.9%, including 5 mucinous and 3 signet cell) and female predominance (21/35, 60%). MSS tumors were left-sided (21/36, 58.3%) with less poorly differentiated tumors (6/36, 16.7%, 2 mucinous) and male predominance (23/36, 63.9%). Comparison of mucin expression revealed greater expression of MUC2 (p=0.007), MUC4 (p=0.014), MUC5AC (p=0.001), and MUC6 (p=0.002) in MSI vs. MSS CRC, but no significant difference in MUC1. The differences in MUC2, MUC4, and MUC5AC remained after statistically controlling for gender and tumor location. Since no MSS tumors were positive for MUC6, analysis could not be done. Mucinous and signet cell CRC were positive for MUC2 (10/10), MUC4 (8/10), MUC5AC (6/10), and MUC6 (2/10), while other tumors showed far less staining. There was no significant correlation between any mucin and CDX-2.
**Conclusions:** MSI CRC showed increased MUC2, MUC4, MUC5AC and MUC6 compared to MSS CRC. Since the differences remained between the MSI and MSS tumors when gender, location, and age were controlled, the differences may be associated with either tumor differentiation and/or MSI status.

### 461 Prognostic Significance of COX-2 and VEGF Expression in Human Rectal Cancer Treated with Pre-Operative Radiotherapy

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**Background:** A specific cause-effect relationship between the overexpression of COX-2 and colorectal carcinogenesis by promoting tumor angiogenesis has been demonstrated in recent studies. VEGF is the most potent mitogen for vascular endothelial cells as well as an important mediator of the COX-2 angiogenic pathway. Increasing effort direct toward searching specific molecular targeted therapies are done. The aim of this study was to determine the prognostic value of the expression of COX-2 and VEGF in a group of patients with locally advanced rectal cancer treated with preoperative radiotherapy and chemotherapy following by radical surgery to identify a subgroup of high risk patients which could benefy from selective treatment.
**Design:** Eigty-three pretreatment diagnostic biopsies were analysed. Inmunohistochemical study in each sample was performed by COX-2 (4H12, Novocastra 1:100 dilution) and VEGF (A-20, St. Cruz Biotech. 1.200 dilution). The immunostained specimens were independly evaluated by two blinded investigators. The extend of COX-2 and VEGF staining was recorder using a 3-grade system base on the percentage of tumor epithelial cell stained: grade 0: 0-10%, grade 1: 10-20% grade 2: 20%- 70% and grade 3: 70%-100% .The longrank test and proportional hazards regression analysis were used to calculate the probability of COX-2 and VEGF expression associated with pathological local recurrence and survival.
**Results:** Preoperative treatment resulted in pathological complete remission in 8 cases (10%) and downstaging in 38 cases (46%)A total of 38 tumors showed COX-2 expression (46%) and 40 tumors demonstrated VEGF expression (48%). There was no association with response to radiotherapy. There was a significant correlation between positive expression of both biomarkers (p:0.031). Disease-free survival was reduced among patients with VEGF expression ($p<0.05$) but this result was not observed with COX-2 expression.

**Conclusions:** 1) COX-2 and VEGF are expressed in a significant number of rectal tumors. 2)Only VEGF positive expression but not COX-2 expression is an indicator for low disease-free survival. 3) Immunohistochemical analysis of VEGF expression in pretreatment biopsies may identify a group of high-risk patients with rectal cancer which could benefy from novel therapeutic modalities against this angiogenic factor.

### 462 Duodenal Biopsies with Increased Intra-Epithelial Lymphocytes in the Absence of Villous Blunting

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**Background:** The isolated finding of increased intraepithelial lymphocytes (IEL) in a duodenal biopsy, while non-specific, is a manifestation of several pathologic conditions including celiac disease(CD)(AJG – Vol. 98, No. 9, 2003, p 2027-33). The aim of this study was to explore the significance of this finding in an urban academic hospital.

**Design:** A retrospective 3 year clinical record and laboratory data review of pts with duodenal biopsy diagnoses of increased IELs with no other duodenal histologic alterations and no prior history of CD.

**Results:** Of the 31 pts 10 were pediatric with an avg. age of 10 yr(range 2-17 yr), F:M of 2.3:1, 70% were white(7) and 30% black(3). The 21 adult pts had an avg. age of 47yr(range 19-78 yr), F:M 2:1, 67% were white(14), 14% black(3), 2% Hispanic(1) and unknown in 14%(3) . The presenting clinical complaints were: pediatric pts – abdominal pain(7), diarrhea(3), GERD(2), failure to thrive(1), anemia(1), vomiting(1); adult pts - abdominal pain(5), GERD(5), diarrhea(5), dyspepsia(3), vomiting(3), anemia(2). Endoscopically the duodenum was normal in all but one case (friable mucosa). The specific cause of the presenting complaints was established in 20 pts: H pylori gastritis (HPG) 25%(5), CD 15%(3), GERD 15%(3), Crohn's disease (CRD)10%(2), lymphocytic colitis (LC)10%(2), irritable bowel syndrome (IBS)10%(2), ulcerative colitis 5%(1), acute self-limited colitis 5%(1). No pts were immunocompromised, 1 pt had NSAID use and there were no cases of giardiaisis. Three pts had sarcoidosis, 2 pts were HIV positive, and one case each of eosinophilic syndrome, Lyme disease, rectal carcinoid, type 1 and type 2 diabetes mellitus and one pt with both hepatitis B and C infection. In 12 of 31 biopsies CD serologies were done, including all 3 CD cases. There was no difference in CD serology ordering frequency whether CD was mentioned as an etiologic possibility in the pathology note (40% vs. 33% when not noted). In 2 of the 3 CD cases the serologic results were known and both were positive for anti-gliadin antibodies(AGA) and negative for endomysial antibodies (EMA). Ordering pattern included EMA and AGA in combination (7/12), tissue transglutaminase (TTG) in 4/12, and in 1 case AGA alone. There were no positive EMA or TTG, and one falsely positive AGA.

**Conclusions:** Isolated IELs in a duodenal biopsy, while non-specific, is associated with a myriad of clinical presentations and diseases. HPG, CD, CRD and LC compose the majority of these associated diseases and each should be carefully excluded when confronted with this biopsy finding.

### 463 Immunostaining for AMACR Is Useful in Distinguishing Reactive from Dysplastic Epithelium in Barrett's Esophagus and Inflammatory Bowel Disease

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**Background:** Alpha methylacyl-CoA racemase (AMACR) catalyses the racemization of alpha-methyl branched carboxylic coenzyme A thioesters and is overexpressed in a variety of neoplasms, such as prostate and colon cancer. The aim of this study was to evaluate AMACR expression in the metaplasia-dysplasia-carcinoma sequence in Barrett's esophagus (BE) and inflammatory bowel disease (IBD) and to determine if its expression can be used to distinguish negative (reactive) from dysplastic epithelium in these conditions.

**Design:** 195 routinely processed biopsy and/or resection specimens [256 foci; 80 with intestinal metaplasia (IM), 27 indefinite for dysplasia (IND), 43 low-grade dysplasia (LGD), 66 high-grade dysplasia (HGD), and 40 invasive adenocarcinoma (ACA)] from 134 patients with BE (M/F ratio: 5.7, mean age: 67 yrs) and 106 specimens (150 foci; 35 negative, 14 IND, 60 LGD, 24 HGD, and 17 ACA) from 59 patients with IBD [43 with ulcerative colitis (UC), 16 with crohn's disease (CD); M/F ratio: 1.7, mean age: 56 yrs] were immunostained with a monoclonal AMACR antibody (p504S). The degree of staining in all cases was graded in a blinded fashion as follows: 0 = 0% cells positive, 1+ = 1-10% positive, 2+ = 10-50% positive, or 3+ = >50% positive.

**Results:** In patients with BE, AMACR was not expressed in any foci with reactive IM only (0%) but was significantly increased ($p<0.001$) in foci of LGD (46%) (grade 1: 21%, 2: 16%, 3: 9%), HGD (76%) (grade 1: 17%, 2: 32%, 3: 27%) and ACA (75%) (grade 1: 18%, 2: 20%, 3: 38%). 9/26 (33%) IND foci, from 6 patients, were positive focally (grade 1: 18%, 2: 15%). However, 3 of these 6 patients had follow-up information available, and all had developed LGD or ACA. Similarly, in patients with IBD, AMACR was only expressed in one UC foci negative for dysplasia (grade 1: 2.9%), but was significantly increased ($p<0.001$) in foci of LGD (87%) (grade 1: 6.7%, 2: 25%, 3: 55%), HGD (79%) (grade 1: 4%, 2:17%, 3: 58%), and ACA (76%) (grade 1: 0%, 2: 18%, 3: 59%). Only 3/14 (21%) IND foci, from 2 patients, were focally positive (all grade 1). However, both these patients had dysplasia upon follow-up.

**Conclusions:** AMACR is involved in the neoplastic progression of BE and IBD. The high degree of specificity of AMACR for dysplasia/carcinoma in BE and IBD suggests that it may be useful to distinguish reactive from neoplastic epithelium in these conditions.

### 464 Contemporary Morphologic Features of Ileal Crohn's Disease and Definition of Backwash Ileitis Using Archival and Current Colectomy and Biopsy Specimens

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**Background:** The morphologic features of terminal ileal (TI) involvement by Crohn's disease (CD) and backwash ileitis (BI) in ulcerative colitis (UC) are vague, partly because they have changed over time with improved medical therapy and earlier diagnosis. We studied archival and contemporary specimens of CD and UC patients to clarify the morphology of these two entities.

**Design:** TI sections from 25 colectomies from patients with fulminant UC during 1970 – 1972 and 25 colectomies removed for steroid dependence/ anemia during 2001-2004 were reviewed. Terminal ileum biopsies from 100 active CUC and 100 CD patients during 1-7/2004 were reviewed. Morphologic features recorded included extent of erosions, microscopic foci of activity, and presence of active inflammation admixed with lamina propria edema. All CD cases had radiographic and/ or endoscopic TI involvement.

**Results:** 1970's fulminant UC resections: All 25 had active enteritis in the distal 3 cm of the TI. 20 cases (80%) had erosions in the distal 2 cm. Active enteritis was moderate/ marked and confluent in the distal 1 cm and tapered to none/ minimal at 3 cm proximal. No cases had mucus gland metaplasia.

2000's UC resections: 4 cases (16%) had focal mild activity involving 1-3 adjacent villi and the subjacent lamina propria without erosions. These foci were not associated with edema, mucus gland metaplasia, or branched crypts.

Contemporary active CUC TI biopsies (mean, 2.2 fragments/case): 6 (6%) had minimal neutrophilic inflammation in the stroma of one or two villi unassociated with surface epithelial changes, edema, erosions, mucus gland metaplasia, or crypt branching in one biopsy fragment.

Contemporary CD TI biopsies (mean, 3.4 fragments/ case): 97 (97%) had one to six foci of activity/ tissue fragment in most or all tissue fragments admixed with edema. Of these, 37 had focal erosions and 44 had villous architectural changes or lymphoid aggregates surrounded by edema. 3 cases had normal TI biopsies. None had granulomas.

**Conclusions:** The morphology of CD in TI biopsies is multiple foci of active inflammation surrounded by edema, often with lymphoid aggregates. A small minority of contemporary CUC patients have isolated small foci of neutrophils in the lamina propria unassociated with other changes. Although BI was common in fulminant UC of prior eras, it is not seen in contemporary UC patients. Multifocal mild active enteritis with edema are the characteristic features of CD, not UC with BI in contemporary IBD patients.

### 465 Chromosome and Gene Copy Alterations in Barrett's Esophagus (BE)-Associated Superficial Esophageal Adenocarcinoma: Fluorescence In Situ Hybridization (FISH) Study

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**Background:** Previous conventional cytogenetic and comparative genomic hybridization studies have shown several frequent genomic aberrations occurring in BE-associated esophageal adenocarcinoma. Specific genomic abnormalities may be related to biologic behavior of these neoplasms and their prognosis. In this pilot study, we assessed the frequency of such changes as detected by FISH in superficial tumors treated by esophagectomy at our institution.

**Design:** We studied 20 esophagectomy specimens containing superficial (submucosal [T1] or intramucosal) carcinomas arising in the setting of BE. Tissue microarray was constructed and array sections were analyzed by multi-color FISH utilizing locus-specific probes (LSI) to *EGFR* (7p12), *C-MYC* (8q24), *HER2* (17q12) and *TOPO-2-Alpha* (17q21) genes, as well as their corresponding centromeric probes (CEP) 7, 8, and 17, respectively. A positive FISH result was defined as presence of greater than 30% of tumor cells with gain or loss of the LSI signals compared to their corresponding CEP signals (gene amplification or deletion) or synchronous gain of both CEP and LSI signals (chromosomal gain).

**Results:** Gene amplification for *C-MYC*, *HER2* and *EGFR* was detected in 5/20 (25%), 4/18 (22%) and 0/19 (0%) cases, respectively. *TOPO-2-Alpha* gene deletion was detected in 1 of 19 (5%) cases. Chromosomal gains were seen in 9/19 (47%), 5/20 (25%) and 18/18 (100%) with CEP probes to chromosomes 7, 8 and 17, respectively.

**Conclusions:** When applied to routine esophagectomy specimens, FISH detects chromosomal aneusomy and/or gene amplification in a substantial proportion of superficial BE-associated esophageal carcinomas. Future study of a greater cohort of patients will address the potential significance of genomic abnormalities for the prognosis of superficial tumors.

### 466 Differential MLH1 Promoter Hypermethylation in the Identification of Sporadic Mismatch Repair-Deficient Colorectal Cancers

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**Background:** CpG island methylation has been associated with alterations in chromatin structure and transcriptional repression. Promoter hypermethylation of the DNA mismatch repair gene MLH1 has been implicated in a subset of colorectal cancers (CRC) which show loss of expression of MLH1. Pre-neoplastic epigenetic alterations have also been documented in the colon. We investigated the relationship between MLH1 promoter hypermethylation in tumor and paired non-neoplastic mucosa, and MLH1 expression in a series of colorectal cancers.

**Design:** This study included 94 cases of colorectal cancer. DNA was extracted from snap-frozen paired tumor and histologically normal mucosa from each case. The

methylation status of the MLH1 promoter was investigated in each case using methylation-specific PCR for tumor and normal DNA. MSI testing was performed using the National Cancer Institute consensus panel of five microsatellite markers. Tumors displaying instability in at least 2 of the 5 markers were regarded as exhibiting high-level microsatellite instability (MSI-H). MLH1 expression was assessed by immunohistochemistry

**Results:** Promoter hypermethylation of MLH1 was detected in 13 of 94 (14%) tumors and in 3 of 94 (3%) paired normal mucosa samples. Of the tumors exhibiting MLH1 promoter hypermethylation, 7 displayed loss of MLH1 expression by immunohistochemistry and were MSI-H. The remaining 6 tumors neither displayed loss of MLH1 expression nor were MSI-H. MLH1 promoter hypermethylation was detected in the normal mucosa from 3 out of these 6 cases. Such concomitant promoter hypermethylation tended to be associated with retention of MLH1 expression and microsatellite-stable status.

**Conclusions:** Relative levels of promoter methylation between the tumor and the normal mucosa may be the determining factor in the control of MLH1 expression. Tumor hypermethylation may only be significant when there is a differential with the methylation status of the normal mucosa. Assessing the methylation status of paired normal mucosa may therefore be of value in clarifying the phenotype of some colorectal cancer patients.

### 467 Immunohistochemical Staining for MLH1, MSH2 and MSH6 Identifies Germline Mutations in Mismatch Repair Genes in Colorectal and Endometrial Cancers Initially Found To Be Microsatellite Stable

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**Background:** The detection of microsatellite unstable (MSI+) colorectal or endometrial carcinoma is vital for prognostic and possibly therapeutic purposes and to help screen for Hereditary Nonpolyposis Colorectal Cancer (HNPCC). MSI analysis and immunohistochemistry (IHC) are approaches to detect patients most likely to have MSI+ tumors and possibly germline HNPCC mutations, but which is the most efficient and practical approach is controversial. We determined the IHC pattern of expression for MLH1, MSH2, MSH6 in colorectal or endometrial cancer patients meeting modified Bethesda criteria for HNPCC but with tumors found to be microsatellite stable (MSS) using MSI analysis.

**Design:** All patients diagnosed with colorectal or endometrial cancer since 1/99 were offered participation in the Columbus-area HNPCC study regardless of age or family history. Tumors were evaluated for MSI using a modified Bethesda panel of microsatellite markers (BAT25, BAT26, D18S69, D2S123, D5S346). All patients found to be MSS by 8/1/02 and meeting any of the following criteria were evaluated; diagnosis under age 50, synchronous or metachronous colon and or endometrial cancers, or a first degree relative with colon or endometrial cancer. IHC for MLH1, MSH2, and MSH6 was performed. Greater than 1% staining was considered positive and staining in all negative cases was repeated.

**Results:** 154 MSS patients met criteria and had sufficient tumor for staining. Staining failed for all proteins in 1 case and MSH6 failed in 13. Of the 153 tumors, 6 showed loss of protein expression; MLH1 was lost in 3, MSH6 in 2, and MSH2 and MSH6 in 1 case. Interestingly 4 of these 6 cases were mucinous tumors. Germline DNA from all 6 patients underwent full sequence analysis and MLPA for large rearrangments (for *MLH1* and *MSH2*) of the gene implicated by IHC results. Two germline mutation were found, one each in *MSH2* and *MLH1*. MSI testing was repeated in 5/6 cases and 3/5 were MSI-high on repeat analysis using a different area of tumor.

**Conclusions:** IHC confirmed microsatellite status in 96% of MSS cases. Two (1.3%) new cases of HNPCC were diagnosed among a high risk cohort of 154 patients whose tumors had been found to be MSS. A potential pitfall in MSI analysis may be mucinous tumors, either due to insufficient cells or interference with MSI analysis. In patients with a suspicious history who have MSS mucinous tumors, IHC may be helpful in addition to MSI analysis.

### 468 Role of Serologic Markers in Diagnosis of Inflammatory Bowel Disease

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**Background:** The diagnosis of inflammatory bowel disease (IBD) requires a combination of clinical, endoscopic, and complex pathologic evaluations. Non-invasive laboratory tests, such as serological tests for atypical p-ANCA and anti-*saccharomyces cervisiae* antibodies are now being suplemented with new markers, such as NSNA (neutrophil specific nuclear antibody by ELISA), and tests for antibodies to bacterial components such as anti-OmpC IgA.

**Design:** We investigated 71 patients (24 with IBD, 47 negative for IBD), who received a full work-up, serologic testing (Prometheus Laboratories) and endoscopic biopsies within 2 months of serologic testing. Final biopsy diagnoses for IBD were compared to the results of the reference laboratory test panels for IBD screening (qualitative determinations of NSNA, ASCA IgG, ASCA IgA, anti-OmpC IgA) and IBD confirmation (quantitative evaluation of the preceding four markers and p-ANCA evaluation by immunofluorescence). All 71 patients were screened and the 53 samples that screened positive were tested with the confirmatory panel. We analyzed the diagnostic utility of the five components of IBD confirmation panel, and evaluated multivariate models for the interpretation of the 5-test panel. Multivariate analysis was performed using discriminant function analysis (DFA), and artificial neural networks (NN).

**Results:** See tables Nr.1 and 2.

| Test name | p-ANCA | NSNA | IgA ASCA | IgG ASCA | anti-OmpC IgA |
|---|---|---|---|---|---|
| ROC analysis, AUC (area under the curve) | N/A | 0.722 | 0.49 | 0.528 | 0.366 |
| Best cut-off, units | N/A | 20.75 | 13.1 | 16.4 | 8.7 |
| Sensitivity, % | 57.1 | 61.9 | 23.8 | 38.1 | 52.4 |
| Specificity, % | 68.8 | 87.5 | 75.0 | 75.0 | 43.7 |
| PPV, % | 54.5 | 90.4 | 64.5 | 74.4 | 61.7 |
| NPV, % | 71.0 | 54.6 | 34.0 | 38.8 | 30.4 |

| | Reference laboratory interpretation | | Multivariate analysis | |
|---|---|---|---|---|
| Test name | IBD screening panel | IBD confirmation panel | DFA | NN |
| Sensitivity, % | 87.5 | 71.4 | 38.1 | 67.9 |
| Specificity, % | 31.9 | 56.3 | 90.6 | 87.5 |
| PPV, % | 39.6 | 51.7 | 72.7 | 77.9 |
| NPV, % | 83.3 | 75.0 | 69.0 | 80.7 |

**Conclusions:** NSNA alone was as sensitive and specific for the prediction of biopsy-positive IBD as was the 5-test panel. DFA algorithms consistently selected a univariate model involving only NSNA as the best predictor of biopsy status. Multivariate analysis with NN provided only minor improvement compared to the univariate model using NSNA alone. Furthermore, the specificity of NSNA alone (87.5%) was better than that achieved by the reference laboratory's 5-test panel (56.3%). These results question the clinical utility and the cost-effectiveness of using multi-test serological panels for the diagnosis of IBD.

### 469 Claudin 1 and Claudin 4 Expression in Distal Gastric Adenocarcinomas

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**Background:** The claudins comprise a multigene family of integral membrane proteins which play a major role in tight junction formation and function. The extent of claudin-1 and 4 expression has been recently described in both esophageal and colonic but not in gastric adenocarcinomas. The pattern of claudin-1 expression has not been studied in normal gastric mucosa, whereas claudin 4 is expressed in intestinal metaplasia but not in normal gastric mucosa.

**Design:** Tissue microarrays were created from paraffin embedded samples from 135 patients with distal gastric adenocarcinomas. Sixty of these cases were intestinal type and 75 were diffuse or mixed types. In addition to the tumor, cores of normal adjacent gastric mucosa and intestinal metaplasia were retrieved from the majority of cases. Cores from lymph node metastases were also obtained. The microarrays were immunohistochemically stained for claudin 1 (polyclonal rabbit, 1/125, Zymed, Laboratories) and claudin 4 (monoclonal mouse, 1/500, Zymed).

**Results:** Moderate (2+) claudin-1 and focal weak (1+) claudin-4 membranous staining was present in normal gastric mucosa. Areas of intestinal metaplasia were moderately/strongly (2-3+) positive for both claudins. Moderate to strong (2-3+) staining of claudin-1 and 4 was detected in 71% and 63% of the intestinal type and in 42% and 43% of the diffuse/mixed types of adenocarcinomas. This overall decreased claudin staining pattern in the diffuse as opposed to intestinal types was statistically significant ($p=0.02$). No significant difference was detected between the intensity of claudin staining and tumor grade, stage or patient survival. The claudin 1 and 4 staining patterns of the primary tumors and their corresponding lymph node metastases was concordant in 84% and 81% of the tumors respectively.

**Conclusions:** Claudin 1 and 4 are strongly expressed in the majority of gastric intestinal type adenocarcinomas. The observation that claudin 4 is not expressed by normal gastric mucosa suggests its utility as a potential marker for diagnosis or targeted therapy.

### 470 Multilayered Epithelium at the Squamocolumnar Junction Is a Histological Marker for Gastroesophageal Reflux Disease

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**Background:** Our group has described a distinctive type of multilayered epithelium (ME) that exhibits features of both squamous and columnar epithelia, and proposed that ME represents an early, or intermediate, phase in the columnar metaplasia of Barrett's esophagus (BE). The aim of this prospective study was to evaluate the prevalence and specificity of ME in mucosal biopsies of the squamocolumnar junction (SCJ) from patients who had gastroesophageal reflux disease (GERD) with and without BE.

**Design:** During endoscopic examination of the esophagus, we took two biopsy specimens across the SCJ from 27 patients with BE [15 long segment (LSBE) and 12 short segment (SSBE)], 13 patients who had GERD without BE, and 10 controls who had no symptoms or endoscopic signs of GERD. Biopsy specimens were processed routinely and evaluated for the presence of ME at the SCJ, which we correlated with morphologic features of the columnar portion of the biopsies and with clinical features of the patients.

**Results:** We found ME at the SCJ in 27%, 36%, 25%, and 10% of LSBE, SSBE, GERD, and control patients, respectively. Compared to control subjects, the prevalence of ME was significantly higher in all 3 patient groups ($p<0.05$). Of the 10 asymptomatic controls, 5 had intestinal metaplasia (IM) at the SCJ, and 1 of these had ME. None of the 5 controls without IM had ME. In patients with BE, ME was correlated with shorter lengths of BE (2.4 vs. 5.6 cm, $p<0.05$) but with no other clinical feature. In GERD patients without BE, ME always was found adjacent to cardia-type mucosa.

**Conclusions:** ME at the SCJ is a histological marker for GERD with or without endoscopically-apparent BE. The finding that ME can be present even before BE is manifest endoscopically supports our hypothesis that ME represents an early, transitional form of columnar metaplasia.

### 471 Overexpression of Claudin 4 Is a Marker of Columnar Metaplasia in Gastroesophageal Reflux Disease

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**Background:** Controversy exists regarding the nature of the gastric cardia (i.e., whether it represents a normal structure present at birth or an early form of columnar metaplasia secondary to gastroesophageal reflux disease (GERD). The claudins are a family of transmembrane tight junction proteins that regulate epithelial permeability and have been shown to be altered in some cancers and in Barrett's esophagus. We have anecdotally observed altered claudin 4 expression in columnar mucosa in BE. The aim of this prospective study was to evaluate the expression of claudin 4 in GERD patients with and without BE.

**Design:** Routinely processed mucosal biopsies of the squamocolumnar junction from 27 patients with BE [15 long segment (LSBE) and 12 short segment (SSBE)], 13 with GERD, without BE, and 10 normal controls without signs or symptoms of GERD, were immunostained (ABC method) with antibodies against claudin 4 (Zymed Laboratories). The strength (weak,strong) and distribution of staining was assessed in each type of epithelium present in the biopsies [intestinal metaplasia (IM), pure mucinous (cardia-type) epithelium (MUC), and mixed mucous/oxyntic epithelium (MUC/OXYN)].

**Results:** A distinctive type of staining for claudin 4 was observed in areas of IM in BE. This consisted of strong, diffuse membranous staining in crypt and surface epithelium and was present in areas of IM in 100% of BE cases. In addition, strong claudin 4 expression was present in all areas of MUC (without IM) from BE cases. This pattern of staining was not present in any of the normal controls ($p<0.05$), which, instead, showed weak patchy claudin 4 reactivity in surface and glandular epithelium. The SCJ biopsies in GERD patients consisted of MUC epithelium adjacent to the SCJ in all 13 cases with MUC/OXYN epithelium more distally in 4 cases (31%), and IM in 1 case (8%). A "BE-like" pattern of claudin 4 staining was present in MUC epithelium in 58% of GERD cases, and in the one GERD case with IM, but was not seen in MUC/OXYN in any case ($p<0.05$).

**Conclusions:** Tight junction-associated protein claudin 4 is differentially expressed in the development of IM in BE. Increased expression of claudin 4 may be a marker of early metaplastic change in GERD, and supports the theory that MUC ("cardia-type") epithelium at the squamocolumnar junction is metaplastic in origin.

### 472 Clinicopathologic Significance of Isolated Ileal Aphthous Erosions: A Long Term Follow-Up Study

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**Background:** The significance of isolated endoscopic terminal ileal aphthous erosions in minimally symptomatic patients with altered bowel habits is poorly defined. The proportion of patients with these lesions that evolve into typical, full-blown, Crohns disease (CD) is unclear. This study evaluated a series of such patients to clarify this question.

**Design:** 28 patients who underwent colonoscopy for mild bowel habit alterations during 1993-1997 were identified from the consultation files of the author. Slides were reviewed and follow-up was obtained from the computer or primary physician records. All patients endoscopically had normal background ileal mucosa with one to 'several' isolated, punctate, small (1 – 3 mm) aphthous erosions and normal colonic mucosa.

**Results:** 25 patients (89%) were female. Mean patient age at diagnosis was 42.3 years (range, 26 - 56 years). TI biopsies (mean; 3.6 tissue fragments/ case) of the aphthous ulcers showed small erosions involving 1-2 villi and the surrounding/ intervening lamina propria. The erosions were surrounded by active inflammation, and edema. A lymphoid aggregate was usually sub or immediately adjacent. 20 patients had active inflammation and edema without erosions in biopsies from the surrounding, endoscopically normal mucosa. No granulomas were seen. The mean number of foci of active inflammation per tissue fragment was 2.3 (range, 1- 4). Concurrent colon biopsies that included the right colon were normal. Follow-Up: 8 patients (29%) developed typical, ileocolonic CD that was definitively diagnosed a mean of 3.9 years (range, 1.5 - 6.5 years) after sentinel endoscopy. The other 20 patients were free of CD during the mean follow-up period of 6.6 years (range, 3.4 - 9.2 years). All 8 patients who developed into CD had active inflammation and edema without erosions in other biopsy fragments. Four (14%) non-CD patients were taking NSAIDs at sentinel colonoscopy; which was discontinued, and all had normal ileal endoscopy and biopsies when repeated 9 - 17 months later. These four patients all had normal adjacent mucosal biopsies.

**Conclusions:** Approximately 1/3 of patients with isolated terminal ileal aphthous erosions as the sole endoscopic abnormality eventuate into typical ileocolonic CD. Biopsies of the erosions typically show only focal active inflammation, patchy edema, and a lymphoid aggregate. Active inflammation and edema were present in endoscopically normal small bowel mucosa in all patients who developed CD. The interval until typical, ileocolonic CD is established can be long in this group of patients.

### 473 Patterns of Expression of PIGR in Pre-Neoplastic Mucosa and Adenocarcinomas of the Upper Gastrointestinal Tract

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**Background:** The polymeric immunoglobulin receptor (PIGR) transports IgA from the basolateral portion of epithelial cells to the lumen where it is cleaved and a portion released as a secretory component. Prior studies showed a decrease in PIGR expression in colonic adenocarcinomas but it was expressed in some carcinomas of the upper gastrointestinal tract. Since adenocarcinomas of the upper GI tract arise in association with different risk factors, the aim of this study was to determine if PIGR expression is associated with unique sub-groups of upper GI adenocarcinoma.

**Design:** We examined in detail the expression of PIGR in non-neoplastic gastric mucosa and in intestinalized gastric and Barrett's mucosa and compared PIGR expression patterns in tumors of the stomach, gastroesophageal junction (GEJ) without Barrett's esophagus (BE) and esophageal adenocarcinomas (AC) arising in a background of BE. A total of 43 cases were reviewed including 14 cases of primary gastric adenocarcinomas and 27 cases of adenocarcinoma of the gastroesophageal junction of which 13 were associated with Barrett's esophagus. Immunohistochemical stains were performed on representative sections of each case using a mouse anti-PIGR monoclonal antibody (clone COMPO2, NeoMarkers). A semi-quantitative evaluation of PIGR expression was performed (0: less than 10% positive cells, 1: 10-50% and 2: more than 50% positive cells displaying cytoplasmic and membrane-associated immunoreactivity).

**Results:** PIGR was expressed in all cases of IM and only in some non-neoplastic gastric mucosal foci ($P<0.001$). 64% of gastric AC, 78% GEJ AC associated with BE and 40% of the gastroesophageal junction adenocarcinomas without associated BE expressed PIGR, with scores 1 or 2. There was a difference in expression of PIGR in GEJ adenocarcinomas as compared to gastric and Barrett's associated adenocarcinomas ($P<0.05$). There was no correlation of PIGR expression with tumor grade or stage.

**Conclusions:** Expression of PIGR is focal or absent in the non-neoplastic gastric mucosa but it is uniformly expressed in foci of intestinal metaplasia. The finding that PIGR is less commonly expressed in gastroesophageal junction adenocarcinomas that are not associated with Barrett's as compared to adenocarcinomas of the stomach and those associated with Barrett's, suggests that gastroesophageal junction adenocarcinoma may follow a different molecular pathway and may reflect different underlying risk factors.

### 474 Identification of Colorectal Adenocarcinomas with High-Level Microsatellite Instability Based on Age and Histopathologic Criteria

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**Background:** Criteria for microsatellite instability (MSI) testing to rule out hereditary nonpolyposis colorectal cancer were recently updated. The revised criteria include parameters that can be identified by the pathologist during signout leading to reflex MSI testing. The updated criteria recommends MSI testing in colorectal cancer patients who are less than 50 years of age and in patients less than 60 years of age with tumors that are morphologically suggestive of MSI. The aim of our study was to review the performance of these criteria to identify microsatellite unstable colorectal cancers.

**Design:** Forty-eight colorectal cancers in patients less than 50 years of age were identified and an additional four cases were included based on morphological criteria and age between 50 and 60. One block of representative invasive adenocarcinoma was selected in each case. MSI testing was performed with BAT25, BAT26, D2S123, D5S346, and D17S250. Immunostains for MLH1, MSH2, and MSH6 were performed using standard immunohistochemistry. Tumors were classified into MSI-High (2 or more markers positive for MSI), MSI-Low (only one marker positive for MSI) and Microsatellite-stable (MSS) if no MSI was present.

**Results:** Thirty-six out of 48 cases (75%) were MSS and 12 cases were MSI-High (25%). The median age of patients with MSI-High tumors was 41 and of MSS tumors was 41.5 years. Among the MSI-High tumors 50% were MSH2 negative, 40% were MLH1 negative and 10% were MSH6 negative. Among the patients aged 50-59, there was one MSI-High tumor (MLH1 negative), one MSI-Low and two MSS cases. Typical MSI histology was present in 42% of MSI-High and in 25% of MSS tumors (NS) with a PPV of 36% and NPV of 78%, sensitivity of 42% and specificity of 69%. Of interest, the frequency of node metastasis was significantly higher in MSS adenocarcinomas ($P=0.016$).

**Conclusions:** Since the overall reported prevalence of MSI-High in colorectal cancer is approximately 15%, the updated criteria resulted in identification of an increased number (25%) of MSI-High patients. The combination of age and MSI histologic criteria was able to predict the presence of MSI-High tumors in approximately 1/3 of the cases. In addition to directing patients for genetic counseling, identification of a tumor as having MSI has prognostic value, as these tumors are less likely to show lymph node metastasis at the time of diagnosis.

### 475 Expression of Erythropoietin and Its Receptor Increases in Colonic Neoplastic Progression: The Role of Hypoxia in Carcinogenesis

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**Background:** Tissue hypoxia is a characteristic pathophysiologic property of colorectal cancer. This process may also add to the therapeutic problem of solid tumor resistance to chemo- and radiation therapy. Erythropoietin (Epo) expression is induced by tissue hypoxia. Acting via its receptor (EpoR), Epo inhibits apoptosis of erythroid cells and has been shown to rescue neurons from hypoxic damage. Increased Epo and EpoR expression has been recently described in human breast, renal and cervical carcinoma. Given the characteristic tumor diathesis present in majority of colorectal cancers, we examined whether Epo signaling may play a role in colonic neoplastic progression.

**Design:** Expression of Epo and EpoR was examined using immunohistochemistry in 24 cases of primary colorectal and metastatic adenocarcinomas versus adenomas and normal colonic mucosa. Immunohistochemical stains were evaluated semiquantitatively based on a four tiered scale. Based on the combination of extent and intensity of immunoreactivity, an immunostaining score (0-300) was determined for each sample. Expression of EpoR protein and mRNA was examined using Western blot and RT-PCR, respectively, in both normal colonic tissue and carcinoma specimens in 5 cases.

**Results:** Epo expression was sequentially increased in normal colonic mucosa (8.3 ± 5.6, mean ± SEM), adenoma (26.4 ± 9.1), primary carcinoma (96.1 ± 12.8) and metastatic carcinoma (122 ± 51.3). EpoR expression was also sequentially increased in normal colonic mucosa (22.3 ± 11.8), adenoma (108.7 ± 24.2), primary carcinoma (178.7 ± 16.6) and metastatic carcinoma (220 ± 58.3) ($p<0.05$ for all results). Epo and EpoR showed enhanced expression in the areas adjacent to ischemia/necrosis. Western blot and RT-PCR analysis revealed increased EpoR protein and mRNA levels in carcinoma compared to normal mucosal colon specimens. Focal stromal Epo and EpoR immunoreactivity was present in 5 and 6 cases, respectively.
**Conclusions:** The uniform increase of the expression of Epo and EpoR along the colonic neoplastic sequence and further increase in ischemic/necrotic areas indicates that the Epo signaling pathway is an important component in colon carcinogenesis including possible epithelial-stromal interactions.

### 476 Epidermal Growth Factor Receptor (EGFR) in Primary and Metastatic Colorectal Carcinoma

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**Background:** Epidermal growth factor receptor (EGFR) promotes malignant cell proliferation and cancer progression. Cetuximab (Erbitux), a chimeric human-mouse-monoclonal immunoglobulin G1 antibody is used for treatment of EGFR-expressing, metastatic colorectal carcinoma that has progressed following chemotherapy. This study characterizes the new Food and Drug Administration (FDA) approved EGFR monoclonal antibody used in colorectal carcinoma to predict patient response to erbitux.
**Design:** EGFR monoclonal antibody (DakoCytomation: Clone 2-18C9)(DAKO) was used to immunostain colorectal cancer. Frequency of immunopositivity was noted in primary and metastatic carcinoma, compared in matched primary and metastatic carcinoma, and assessed in six different cancers by tissue microarray (TMA). Membranous immunostain was assessed as intensity (0-3+), and scored as estimated percent of cells positive (0=negative, 1=1%, 2=2-5%, 3=6-10%, 4=11-25%, 5=26-50%, 6=51-75%, 7=76-100%).
**Results:** Of 79 colorectal carcinomas (51 primary, 28 metastatic), 69 (87.3%) were EGFR- positive including 45 primaries (88.2%) and 24 metastases (85.7%). Frequency of immunoreactivity between primary and metastatic lesions was similar (p=0.6067). Forty-five (65.2%) of positive tissues immunostained with 3+ intensity and 21 (30.4%) with 2+ intensity. Fifty-seven (82.6%) scored 4-7 (>10%). Expression was strongest in small invasive clusters of carcinoma at the periphery of the primary lesion. The 20 matched primary and metastatic colorectal carcinomas expressed concordant EGFR in 18 (90%), 17 immunopositive, one immunonegative. The two discordant cases, expressed 2+ and 3+ intensity EGFR, score 4, in the primary lesion only. By TMA, 61.5% of 26 ovarian, 84% of 25 endometrial, 19.2% of 26 breast, 64% of 25 lung, 11.1% of 18 melanomas, and 34.8% of 23 colorectal carcinomas were EGFR-positive.
**Conclusions:** Primary or metastatic colorectal carcinoma may be immunostained for EGFR to predict response to erbitux, in view of the high frequency (>85%) of EGFR expression in both, and the high frequency (90%) of concordance in EGFR expression. Small biopsies may be EGFR-immunonegative due to sampling error as suggested by the strong peripheral stain in primary lesions, and the low frequency (34.8%) in TMAs of colorectal carcinoma.

### 477 Vessels' Morphology of Polyps Distinguish *SMAD4* and *BMPR1A*-Related Digestive Juvenile Polyposis Syndrome

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**Background:** Juvenile polyposis syndrome is an hamartomatous intestinal polyposis syndrome associated with malignant change in 20% of cases at an early age. Germline mutations in three genes, *SMAD4*, *BMPR1A* and *PTEN,* have been found in a few number of patients affected by this disease, without any strong phenotype-genotype correlation, that could be predictive of the long term evolution.
**Design:** Thirty unrelated patients affected by juvenile polyposis syndrome were analysed for clinical and histological features and these data were analyzed with respect to the presence of germline mutations in the *BMPR1A*, *SMAD4* and *PTEN* genes.
**Results:** Deleterious alterations were found in 18/30 (60%) patients: 5 on *BMPR1A*, 9 on *SMAD4* and 4 on *PTEN*. No gastric polyps were observed in *BMPR1A* mutation carriers. Low grade adenomas were present in both *SMAD4* and *BMPR1A* mutation carriers (4/9 and 1/5 respectively), *SMAD4* mutated cases only harbouring colon adenocarcinoma lesions (4/9). None of the *PTEN*-related lesions were carcinomatous. Polyps in these patients were predominantly hyperplastic. Malformative vessels within the polyps were specifically found in patients with *SMAD4* mutation.
**Conclusions:** Our study indicates that *SMAD4* germline mutations are associated with the most aggressive digestive phenotype in patients affected by juvenile polyposis syndrome. Polyps developed in this context are identifiable by the presence of malformative vessels.

| Mutated gene | *BMPR1A* | *SMAD4* | *PTEN* | Non-mutated |
|---|---|---|---|---|
| Number of patients | 5 | 9 | 4 | 12 |
| Age (years) | 6-31 | 1-36 | 4-75 | 5-67 |
| Gender<br>Females<br>Males | 3<br>2 | 2<br>7 | 0<br>5 | 8<br>4 |
| Location<br>Stomach<br>Small intestine<br>Colon and rectum | 0<br>1<br>5 | 2<br>1<br>9 | 2<br>2<br>3 | 0<br>0<br>12 |
| Familial history of colorectal cancer | 1 | 1 | 0 | 0 |
| Hamartomatous polyp<br>Epithelial component (% of the polyp surface)<br>Prominent hyperplastic epithelium<br>Stromal component (% of the polyp surface)<br>Malformative vessels<br>Inflammation | 30-80<br>2<br>20-70<br>0<br>1 | 50-95<br>1<br>5-50<br>7<br>5 | 95<br>4<br>5<br>0<br>0 | 50-95<br>9<br>5-50<br>4<br>5 |
| Low grade adenoma | 1 | 4 | 0 | 5 |
| High grade adenoma and/or adenocarcinoma (T1N0Mx) | 0 | 4 | 0 | 0 |

### 478 *CDC2/CDK1* Expression in Esophageal Adenocarcinoma and Precursor Lesions Serves as a Diagnostic and Cancer Progression Marker and Potential Novel Drug Target

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**Background:** Esophageal adenocarcinoma (EA) arises through well-defined precursor lesions, although only a subset of these lesions advances to invasive adenocarcinoma. The lack of markers of progression, presentation at advanced stage, and limitations of conventional chemotherapy result in >90% mortality for EA. To identify potential prognostic markers and therapeutic targets, we compared gene expression profiles from EA cell lines and normal esophageal epithelium using the Affymetrix U133_A gene expression platform. A subset of differentially upregulated genes was validated by immunohistochemistry, including one to which there is a known inhibitor .
**Design:** EA cell lines (BIC1, SEG1, KYAE, OE33) were compared to epithelial scrapings. Fragmented cRNA samples were hybridized to the complete human U133 GeneChip® set (HG_U133 A). Genes expressed ≥threefold in EA *versus* normal were identified. For validation, tissue-microarrays (TMAs) were constructed from resections from 61 EA patients. Cell lines were also treated with the CDC2 inhibitor EM-1421 and analysis of relative fold-change of *CDC2* gene expression in the pre- and post-treatment cell lines was performed by real-time quantitative PCR.
**Results:** There were 560 transcripts with >3 fold upregulation in EA cell lines compared to normal. Using TMAs composed of squamous mucosa (n = 20), Barrett esophagus (BE, n = 10), low grade dysplasia (LGD, n = 14), high grade dysplasia (HGD, n = 27), EA (n = 59), and node metastases (n = 27), we confirmed differential upregulation of 3 proteins (Cdc2/Cdk1, Cdc5, and Igfbp3) in EA and BE lesions. Protein expression mirrored histologic progression for all 3. For Cdc2, 87% of LGD had at least focal surface Cdc2/Cdk1 and 20% had >5% surface staining. 96% of HGD expressed abundant surface Cdc2/Cdk1. EA and metastases had ubiquitous expression. EA cell lines treated with the novel *CDC2/CDK1* transcriptional inhibitor, Tetra-*O*-methyl nordihydroguaiaretic acid (EM-1421), had dose-dependent reduction in cell proliferation, paralleling downregulation of *CDC2/CDK1* transcript and protein levels
**Conclusions:** These findings suggest a role for *CDC2/CDK1* in esophageal adenocarcinogenesis, both as a new histopathologic marker of dysplasia and a putative treatment target.

### 479 Immunohistochemical Expression of the PPM1D/wip-1 Protein Correlates with Aggressive Tumor Behavior in Invasive Colorectal Carinomas (CRCS)

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**Background:** The protein encoded by the *PPM1D/wip-1* gene, which maps to 17q21-q24, is a member of the PP2C family of Ser/Thr protein phosphatases. This enzyme is thought to down-regulate the apoptotic activity of p53, by inactivating p38 MAPK, a kinase involved in p53 phosphorylation of Ser46 and Ser33. Recently, in a mouse mammary tumor model, it was shown that inactivation or depletion of wip-1 phosphatase suppressed tumor formation. Gain in copy number and/or amplification of the *PPM1D/wip-1* gene has been encountered in ovarian clear-cell adenocarcinomas and neuroblastomas and as part of a 17q23 amplicon in breast cancer cell lines. The prognostic significance of over-expression of the PPM1D/wip-1 protein has not been previously studied in colon cancer.
**Design:** Formalin-fixed paraffin-embedded tissue from 99 colorectal adenocarcinomas were immunostained by an automated method (DakoCytomation, Carpinteria, CA) using a new monoclonal anti human wip-1 antibody (Trevigen, Inc., Gaithersburg, MD). Nuclear immunoreactivity was semi-quantitatively scored based on intensity and percentage of positive cells and results were correlated with morphologic and prognostic variables.

**Results:** Immunoreactivity was exclusively nuclear and was positive in 84/99 (85%) of the tumors. Wip-1 correlated with tumor grade (p=0.01), with 9/9 (100%) grade 3, 71/82(86%) grade 2 and 4/8 (50%) grade 1 tumors positive. Wip-1 expression also correlated with advanced tumor stage (p=0.01) with 73/82 (89%) advanced stage compared to 11/17 (65%) low stage. Additionally, wip-1 positivity correlated with lymph node metastases with 41/84 (49%) node positive compared to 2/15 (13%) node negative cases. There was no significant correlation with survival.
**Conclusions:** The expression of the PPM1D/wip-1 phosphatase in invasive CRCs significantly correlates with tumor stage, grade and lymph node metastases. We are currently investigating the prognostic and possible therapeutic significance of the *PPM1D/wip-1* gene expression in CRCs.

### 480 Pathology of the Ileum in Chronic Ulcerative Colitis

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**Background:** Patients with chronic ulcerative colitis (CUC) may develop inflammation in the distal ileum believed to be due to "backwash" of cecal contents ("backwash ileitis"). However, a systematic analysis of ileal changes in CUC has never been performed, and the prevalence and criteria for backwash ileitis have not been defined. The aim of this study was to evaluate the prevalence and spectrum of inflammatory changes in the ileum in patients with CUC.
**Design:** Routinely processed ileocolonic resection specimens from 200 consecutive patients with clinically and pathologically confirmed CUC were evaluated for a wide variety of pathologic features in the ileum and colon. The ileum data was correlated with the clinical features and the pathologic findings in the colon. Follow-up data was obtained (mean follow-up: 32.8 mths) to confirm absence of Crohn's disease (CD).
**Results:** Overall, 34 of 200 (17%) CUC patients had inflammatory changes in the ileum (M/F ratio: 16/18, mean age: 42 yrs). 32/34 (94%) had pancolitis, which was significantly higher than the rate of pancolitis (39%) in patients without ileal disease (N=166) (p<0.001), but there were no other differences between patients with or without ileal pathology. In the colon, 22/34 (65%) patients had severe activity. Ileal changes included villous atrophy and crypt regeneration without increased inflammation (N=3), increased neutrophilic and mononuclear inflammation in the lamina propria (N=6), patchy cryptitis and crypt abscesses (N=21) and focal superficial surface erosions (N=4) some with pyloric metaplasia (N=2/4). In general, the severity of ileal changes paralleled the severity of colonic activity. However, 2/4 (50%) patients with superficial erosions in the ileum had subtotal or left sided colitis only, and had only mild colonic activity. Upon follow-up of patients with erosions (mean: 48.5 mths, range: 26-102 mths) none developed manifestations of CD anywhere in the GI tract.
**Conclusions:** Ileal changes in CUC are not uncommon (prevalence: 17%), are generally mild in nature, (villous atrophy, increased inflammation, scattered crypt abscesses), and are consistent with a "backwash" etiology. However, rarely, ileal erosions may occur in patients without cecal involvement, which may indicate an alternative etiology, such as ileal involvement by CUC.

### 481 *Yersinia* DNA from Gene That Modulates Macrophage Response Is Detected in Intestines of Crohn's Disease Patients

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**Background:** Previously, we detected *Yersinia enterocolitica* (YE) and *Yersinia pseudotuberculosis* (YP) DNA from chromosomally encoded invasion genes in 31% of tested bowel resections from Crohn's disease (CD) patients (AJSP 27: 2003). Recent microbiological studies showed that plasmid-encoded genes known as Yop (YopP for YE, YopJ for YP) are essential to bacteria/host macrophage interactions, and that Yop gene variants are associated with specific high and low virulence *Yersinia* biogroups. As CD is a chronic inflammatory process, understanding macrophage interactions with possible bacterial "triggers" may be critical to understanding persistence of gut inflammation.
**Design:** Eighty-two resection specimens from 82 patients with an established clinicopathologic diagnosis of CD were obtained. DNA was extracted from paraffin block sections of lesional tissue following proteinase digestion. Polymerase chain reaction was performed using novel primer pairs targeting the YE YopP gene and the YP YopJ gene, and samples were then submitted to gel electrophoresis. Sequencing and BLAST analysis were performed to confirm that PCR products matched expected gene sequences.
**Results:** Twenty-four percent (20/82) of cases tested positive for DNA from the plasmid-encoded Yop genes by PCR analysis. Furthermore, of these 20 positive cases, 13 contained YopP DNA corresponding to low virulence YE, no cases contained YopP DNA corresponding to high virulence YE, and 7 cases contained YopJ DNA corresponding to YP, for whom the virulence characteristics are currently poorly understood.
**Conclusions:** A significant percentage (24%) of CD cases contain Yop DNA, indicating that bacterial DNA essential to modulation of macrophage interactions is present in CD cases. Furthermore, 13 of the cases contained DNA from low virulence strains of YE. It has been previously established that low virulent YE expressing the YopP genes detected here lack the ability to effectively induce macrophage apoptosis, and thus may be able to reside within macrophages for an extended period of time. These preliminary data demonstrating Yop DNA in CD patients suggest that *Yersinia* may persist in tissues and cause a plasmid driven, immune-mediated disease process.

### 482 Outcome in Patients with Colorectal Adenocarcinoma in Relation to the Expression of the Growth Factor Pleiotrophin and Its Receptor Anaplastic Lymphoma Kinase

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**Background:** Pleiotrophin (PTN) is a secreted growth factor that can induce epithelial and endothelial cell growth and survival as well as tumor angiogenesis and metastasis. The ALK tyrosine kinase receptor is a member of the insulin receptor (IR) family due to the high homology of its intracellular kinase domain with IR. The extracellular domain of ALK is a receptor for PTN that transmits the signaling of the growth factor. Overexpression of PTN and ALK has been previously reported in several solid tumors. Here we studied to what extent expression of this growth factor/receptor pair in primary colorectal adenocarcinoma would predict the progression of the disease.
**Design:** Expression of PTN (protein and mRNA) and ALK (mRNA) were evaluated in tissue microarrays (TMAs) of a series of normal colorectal tissues (n=80) and primary colorectal adenocarcinoma (n=147) as well as their liver metastases (n=75) by immunohistochemistry and in situ hybridization, respectively. Expression levels were scored blinded on a four-tier scale and then related to disease stage and patient survival.
**Results:** We found a significant increase in expression of PTN and of ALK in adenocarcinoma and metastasis tissues when compared with normal colorectal tissues (all p<0.001). Positive PTN and ALK were found in the majority of adenocarcinoma and metastases and there was also an increase in overall expression levels in these relative to normal tissues. The highest levels of expression were only found in neoplastic samples. PTN and ALK expression levels were significantly higher in primary adenocarcinoma stage IV then in stage I-III tumors. In addition, low overall survival of patients with non-metastatic disease (stage I III) was significantly related to increased expression levels of PTN and of ALK (p=0.003).
**Conclusions:** We propose that ALK/PTN expression is a prognostic marker of stage I-III colorectal cancer. We hypothesize that therapeutic targeting of this pathway could alter disease progression in colorectal adenocarcinoma that show activation of this pathway and might serve to identify occult metastases in patients.

### 483 Growth Regulation and Apoptosis in Columnar Epithelium underneath Re-Epithelialized Islands of Squamous Mucosa in Barrett's Esophagus

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**Background:** Chronic acid suppression therapy in patients with Barrett's esophagus (BE) often results in the development of re-epithelialized islands of squamous mucosa that cover the underlying BE. Neoplasia can develop in BE located underneath squamous islands (BUSI), which makes endoscopic surveillance difficult. A previous study by our group demonstrated that BUSI shows a significantly lower Ki-67 proliferation rate compared to adjacent areas of BE, particularly in areas of BUSI that do not communicate with the mucosal surface. We postulated that this may be due to altered regulation of cell growth and/or apoptosis. Therefore, the aim of this study was to evaluate markers of cell growth and apoptosis in BUSI.
**Design:** 56 esophageal mucosal biopsies from 25 BE patients (mean age; 64.9 yrs, mean length of BE; 4.8 cm, all on proton pump inhibitors) with BUSI (mean; 2.2 biopsies per patient, range; 1-5) were immunostained for epidermal growth factor receptor (EGFR), transforming growth factor-alpha (TGF-a), bcl-2, and cleaved (activated) caspase-3. The degree and location of staining, and the caspase-3 apoptotic index (AI) was calculated, and the data were compared between BUSI and adjacent BE, and between luminally and non-luminally exposed BUSI, for all parameters.
**Results:** BUSI showed a significantly lower frequency of EGFR (53% vs. 81%, $P < 0.01$) and TGF-a (81% vs. 98%, $P = 0.02$) positivity, and a trend toward a lower frequency of bcl-2 positivity (57% vs. 76%, $P = 0.06$), and AI (0.07% vs. 0.15%, $P = 0.17$), in comparison to adjacent areas of BE. In addition, BUSI that opened directly onto the luminal surface of the mucosa, by either penetrating through or wrapping around overlying islands of squamous mucosa, showed a trend toward a significantly higher AI compared to BUSI that did not reach the mucosal surface, and, thus, was not exposed to the luminal environment (0.09% vs. 0.00%, $P = 0.06$).
**Conclusions:** BUSI shows decreased expression of growth signaling proteins and a low rate of apoptosis, particularly in areas without communication with the luminal surface. Communication with the lumen and possibly exposure to luminal contents are important for growth regulation in BUSI. Despite a decrease in growth protein expression and proliferation (previously reported), the decrease of apoptosis in BUSI may be a factor in promoting neoplasia.

### 484 Endoscopic Mucosal Resection (EMR): An Improved Diagnostic Procedure for Early Esophago-Gastric Epithelial Neoplasms

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**Background:** EMR is advocated for the treatment and staging of superficial esophago-gastric neoplasms. However, recent series reveal disappointing rates of positive margins. In addition to its utility as a staging tool, we hypothesize that EMR may serve as a diagnostic modality. This study compares EMR specimens with endoscopic biopsies for the diagnosis of esophago-gastric superficial neoplasms in correlation with the extent of biopsy sampling.
**Design:** 31 gastric and 10 esophageal EMRs [6 low grade dysplasias (LGDs), 12 high grade dysplasias (HGDs), 21 intramucosal adenocarcinomas (IMCs), 2 submucosal invasive adenocarcinomas (Inv CAs)] were assessed for concordance between pre-EMR biopsies and EMR diagnoses. Discrepancies were considered as either major or minor if the histologic grades differed by 2 or more, or by only one, respectively. Cases

of the "discrepant group" were compared with those of the "concordant group" with regard to the extent of pre-EMR endoscopic sampling determined by calculating the ratio of the mucosal greatest dimension (mm) and /or surface area ($mm^2$) of the lesion (grossly assessed) to the number of biopsy fragments.

**Results:** Of the 41 cases, 16 (39%) had discrepant diagnoses including 14 gastric (45%) and 2 (20%) esophageal neoplasms. A major discrepancy accounted for 2% (n=1, gastric) of the cases. Minor discrepancies were seen in 15 cases (37%). All but 2 of the discrepant cases were found to have higher grade on EMR (Table 1). The average number of biopsy fragments was 4.4 in both concordant and discrepant groups. The maximal dimension (MD), surface area (SA), MD / biopsy fragment (#frgt) and SA / #frgt ratios were significantly greater in the discrepant group than in the concordant group (Table 2).

**Conclusions:** EMRs, frequently revealing higher neoplastic grade, are superior to biopsies for grading of superficial esophago-gastric tumors, particularly for large (>17 mm) lesions. EMR can substantially modify therapeutic decisions, avoiding under- and over-treatment, since HGDs or carcinomas are frequently treated by surgery while LGDs are not. Extensive biopsy sampling may limit the discrepancy.

Table 1

| Bx. \ EMR | LGD | HGD | IMC | Inv CA |
|---|---|---|---|---|
| LGD | 5 | 4 | 0 | 0 |
| HGD | 1 | 7 | 9 | 1 |
| IMC | 0 | 1 | 12 | 0 |
| Inv CA | 0 | 0 | 0 | 1 |

Table 2

| | MD (mm) | MD/#frgt | SA (mm2) | SA/#frgt |
|---|---|---|---|---|
| Discrepant (mean) | 17.3 | 5.0 | 239 | 66.8 |
| Concordant (mean) | 10.4 | 2.8 | 96 | 20.1 |
| P value (Mann-Whitney U) | 0.016 | 0.022 | 0.040 | 0.014 |

### 485 Hereditary Diffuse Gastric Cancer: Genetics, Pathology, and Management

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**Background:** Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant cancer susceptibility syndrome characterized by early onset diffuse (signet ring) carcinomas of the stomach and lobular breast cancers (LBCs). We previously identified germline E-cadherin (CDH1) mutations in 40% of families with a history of multiple gastric cancers and at least one verified DGC in an individual less than 50 years old. Management options for mutation carriers include endoscope screening or prophylactic gastrectomy. Occult early diffuse gastric cancers have been described in all of 12 published prophylactic gastrectomy specimens.

**Design:** To test the sensitivity our screening criteria we ascertained twelve new families who met the above criteria, six early onset DGC cases, and one case of synchronous DGC and LBC and screened them for E-cadherin germline mutations by dHPLC and automated sequencing. To assess the benefit of prophylactic gastrectomy as a risk reduction strategy, clinical data from mutation positive families is being databased.

**Results:** Four of twelve HDGC families, two of 6 early onset diffuse gastric cancer cases (both under 30 years of age) and a single individual with a history of both DGC and LBC proved positive for E-cadherin germline mutation. Two of the families had a C1003T nonsense mutation that has been previously described in a Swedish family. The mutation occurred on a different haplotype in each family thus represents a mutation hotspot rather than a founder mutation.

Four new prophylactic gastrectomies from mutation positive, asymptomatic, endoscopically normal individuals from two families were performed in 2004. All specimens were analyzed in their entirety. Three of the four patients (aged 54, 35 and 32) had multifocal superficial DGC and the fourth (aged 50) had no invasive cancer. Concurrent LBC was detected by preoperative MRI in one individual.

**Conclusions:** In addition to those meeting the family based testing criteria, germline E-cadherin mutation screening may be warranted for individuals with DGC age 30 or less, or a history of both DGC and LBC. The finding of occult invasive DGC in the face of negative endoscopy in three of four new prophylactic gastrectomy specimens highlights the role of this procedure in the management of HDGC.

### 486 Immunohistochemical Expression of *hMLH1*, *hMSH2*, and Ki-67 in Serrated and Neoplastic Lesions in Patients with Inflammatory Bowel Disease

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**Background:** Inflammatory bowel disease (IBD) is associated with an increased risk of colorectal carcinoma (CRC). Chromosomal instability and microsatellite instability (MSI) are both thought to play a role in the pathogenesis of colitis-associated colon cancers. In patients with IBD, colon cancers, dysplasias and regenerative lesions with MSI are more often reported to be of the MSI-low phenotype. The MSI-high phenotype, seen in hereditary nonpolyposis colon cancer-associated CRC and 15% of sporadic CRC, usually results from inactivation of the DNA mismatch repair (MMR) genes, hMLH1 or hMSH2. The serrated neoplasia pathway, which includes sessile serrated polyps, mixed polyps and serrated adenomas, have also been shown to be associated with the MSI-low phenotype. Serrated lesions also occur commonly in patients with inflammatory bowel disease, but how these lesions should be classified with regard to neoplastic risk remains uncertain. We examined sporadic serrated lesions and IBD-associated serrated and neoplastic lesions for expression of hMLH1, hMSH2, and Ki-67 to determine the role of markers traditionally associated with MSI-high neoplasms.

**Design:** Paraffin-embedded sections of 44 lesions, including aberrant crypt foci, hyperplastic polyps, sessile serrated polyps, a serrated adenoma, dyplastic mucosa, and carcinomas, in forty patients with IBD, were immunostained with MLH1 mouse monoclonal antibody (BD Pharmingen, 1:100), Ki-67 mouse monoclonal antibody (Dako, 1:50) and MSH2 mouse monoclonal antibody (Oncogene Research Products, 1:100).

**Results:** All (100%, 44/44) lesions showed expression of MLH1 and Ki-67. Almost all (95%, 42/44) lesions showed staining for MSH2. The two (5%, 2/44) lesions that had loss of staining for MSH2 were a right-sided carcinoma and a left-sided dysplasia in two ulcerative colitis patients. None of the sporadic serrated lesions showed loss of hMSH2 or hMLH1 expression.

**Conclusions:** Serrated lesions in patients with IBD are similar to sporadic serrated lesions in that they retain expression of the MMR genes, hMLH1 and hMSH2, and expression of Ki-67. This is in concordance with the association of IBD and serrated lesions to an MSI-low phenotype and methylation abnormalities. The mismatch repair proteins, hMLH1 and hMSH2, may not play a significant role in most IBD-associated dysplasias and serrated lesions. Instead, methylation of other genes may be of importance in these patients.

### 487 Normal Variation in Intraepithelial Lymphocytes of the Terminal Ileum

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**Background:** Intraepithelial lymphocytes (IELs) are increased in the terminal ileum of patients with immune-mediated inflammatory diseases of the colon, such as lymphocytic and collagenous colitis. However, data regarding the number of IELs present in the ileal mucosa of patients with other chronic inflammatory disorders confined to the colon, including ulcerative colitis (UC) and Crohn's colitis (CC) with ileal sparing, are lacking. Moreover, one frequently encounters increased IELs in ileal biopsies from patients without histologic evidence of overt colitis. In this situation, the clinical importance of mild inflammation in the ileal epithelium is unclear because baseline data defining the number of IELs normally present in the ileum have not been elucidated. The aim of this study was to establish the acceptable range of IELs present in biopsies of normal ileal mucosa and in biopsies from patients with UC and CC with ileal sparing.

**Design:** We evaluated ileal mucosal biopsies obtained during routine screening colonoscopy of asymptomatic patients without colitis (n=71) and biopsies from patients with clinically and pathologically confirmed active UC (n=15) and active CC with ileal sparing (n=14). Immunostains for CD3 were performed on routinely processed 5 µm tissue sections using the standard ABC technique and the number of CD3+ lymphocytes present in three well-oriented villi was evaluated. The average number of CD3 + lymphocytes/100 enterocytes in the three groups was compared statistically.

**Results:** The clinical features of the three patient groups were similar. For asymptomatic patients without colitis, the mean age was 44 years (range: 10-84, male/female ratio: 30/41), compared to 40 years for UC patients (range: 14-61, male/female ratio: 9/6) and 48 years for CC patients (range: 34-61, male/female ratio: 5/9). The mean number of ileal CD3+ cells /100 IELs in asymptomatic patients without colitis was 4 (range: 0-11), compared to 6 (range: 2-18) in UC patients (p=0.015) and 5 (range: 2-15) in CC patients (p=0.021).

**Conclusions:** Occasional IELs are a normal finding in ileal biopsies from asymptomatic patients without colitis. The number of ileal IELs is slightly increased in patients with inflammatory bowel disease confined to the colon, but this finding has no clinical relevance. Therefore, one should avoid overinterpreting the clinical importance of occasional IELs in ileal biopsies from these patients.

### 488 Mastocytic Enterocolitis: Another Possible Cause for Intractable Diarrhea in Adults

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**Background:** In some cases of adult-onset intractable diarrhea, the etiology remains elusive even after repeated laboratory tests, upper and lower endoscopic examinations and intestinal biopsy evaluations. These cases are then clinically suspected as possible variants of irritable bowel syndrome (IBS). In some of such cases, the intestinal mucosa shows increased mast cells and the majority of these patients show resolution of diarrhea when drugs affecting mast cell mediators are given. Mast cell mediated hypersensitivity may be playing a central role in some cases of intractable diarrhea in adults.

**Design:** From 2001-2004, we identified 41 cases (ages 21-65, 25 females and 16 males) who had chronic non-bloody diarrhea, negative laboratory work-up, unremarkable upper and/or lower endoscopic examination and normal duodenal and/or random colonic biopsies on routine H&E stains. Toluidine blue and mast cell tryptase immunostain, were performed on the biopsies and mast cells were counted per high power field (hpf), in the lamina propria. Patients with >20/hpf mast cells were given drugs affecting mast cell mediators - initially ranitidine (H2 receptor inhibitor) and cetirizine (H1 receptor inhibitor) and later, if necessary, cromolyn (mast cell mediator release inhibitor) and followed-up clinically. Similar mast cell stains and counts were performed on normal controls (50) and some patients with identifiable causes of diarrhea such as celiac disease (35), Crohn's (9), and ulcerative (12), collagenous (4) and microscopic colitis (3).

**Results:** Mast cell tryptase immunostain consistently identified greater numbers of mast cells than toluidine blue (>2-3 times) hence it solely was used as a parameter of increased mast cells. Since only 1/50 normal controls had mast cells over 20/hpf, >20/hpf was considered as elevated count. In 24/41 cases (59%) of intractable diarrhea, mast cells were elevated and no patient had systemic mastocytosis. 15/24 patients (63%)

showed significant reduction or cessation of diarrhea on the specified therapy. No patient with other identifiable cause of diarrhea had elevated mast cells.
**Conclusions:** Mastocytic enterocolitis should be recognized as possible cause of intractable diarrhea in some cases mimicking IBS. While biopsies from these patients may show normal duodenal or colonic mucosa on routine staining, in over half of the patients increased mast cells (>20/hpf) may be demonstrated by mast cell tryptase. More than half of such cases respond to one or more of the drugs affecting mast cell mediators.

### 489 Evaluation of Microsatellite Instability, hMLH1 Expression and hMLH1 Promoter Hypermethylation in 176 Colorectal Carcinomas

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**Background:** About 15% of all colorectal carcinomas (CRCs) demonstrate high levels of microsatellite instability (MSI-H) and are currently best identified by molecular analysis of microsatellite markers. Most sporadic CRCs with MSI-H are known to be associated with the methylation of the hMLH1 promoter. Promoter methylation coincided with lack of hMLH1 expression.
**Design:** We aimed to investigate the association between MSI status, hMLH1 protein expression and methylation status of the hMLH1 promoter, and to determine the usefulness of each method in defining the MSI phenotype in sporadic CRCs. We extracted genomic DNA from 176 colorectal carcinomas and corresponding non-neoplastic tissue. MSI testing was performed by PCR using five standard MSI markers (D2S123, D5S346, D17S250, Bat-25, and Bat-26). hMLH1 promoter analysis was investigated by methylation specific PCR (MSP), and expression of the MMR genes hMLH1 and hMSH2 was examined by immunohistochemistry (IHC) using the method of tissue microarray.
**Results:** Of the 176 colorectal carcinomas, 90% were MSS and 10% MSI-H. hMLH1 protein expression was seen in 46.7% MSS tumors, but was related with loss of expression in MSI-H tumors. In the study of methylation PCR for hMLH1 promoter, 26.5% MSS cancers were methylated whereas 80% MSI-H cancers were methylated. MSI status (MSI-H tumors) is related with the methylation of the hMLH1 promoter and loss of MLH1 expression. All colorectal carcinomas were expressed hMSH2.
**Conclusions:** hMLH1 promoter methylation analysis or immunohistochemistry alone cannot replace MSI testing. We suggest that phenotypic evaluation of CRC is performed most reliably with MSI testing, although protein expression analysis and investigation of the promoter methylation status may complement the screening process.

### 490 Detection of Ganglion Cells by RET Oncoprotein in Hirschsprung Disease in Colonic Biopsies and Resections

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**Background:** The absence of ganglion cells (GC) is the primary anatomic abnormality in Hirschsprung Disease (HD), a common disorder affecting infants, resulting in bowel obstruction. Despite the availability of special techniques such as acetyl cholinesterase histochemistry (ACH), S-100 protein and neuron specific enolase (NSE) immunohistochemistry, examination of serial H&E stained sections remains the standard approach in establishing this diagnosis. However, documenting aganglionosis is often difficult on routine H&E sections, ACH histochemistry is technically challenging, and S-100 and NSE staining lack sensitivity and specificity. Retinoblastoma oncoprotein (RET) has been reported to be highly expressed in normal enteric GC. In an effort to find a more sensitive and dependable marker for GC, this study was carried out to compare RET immunoreactivity and routine H&E light microscopy in identifying GC in colonic biopsies and resections for HD.
**Design:** Two hundred and ninety five paraffin embedded tissue blocks from biopsy and resection specimens representing one hundred and thirty one patients with a clinical suspicion of HD were available for inclusion in the study. The presence of remaining tissue in the blocks and the location and depth of the biopsies were used as inclusion criteria. Fifty two blocks demonstrating GC and forty blocks lacking GC on original H&E sections were evaluated for RET immunoreactivity to identify GC. To control for variation in GC population in the blocks, H&E sections immediately preceding the sections evaluated for RET immunoreactivity were used for baseline morphologic examination.
**Results:** All forty blocks showing GC on H&E were also positive for GC on RET staining (100%). Forty-eight out of fifty one blocks negative for GC on the H&E (94%), did not demonstrate GC on RET staining. However, three blocks negative for GC on H&E (6%), showed GC on RET stained sections. One block demonstrated GC on both the RET and adjacent H&E sections, although the original H&E levels were negative for GC.
**Conclusions:** RET immunoreactivity has comparable specificity but slightly higher sensitivity than routine H&E staining in the identification of GC in biopsy and resection specimens from patients with a clinical suspicion of HD. GC are more readily identified by RET immunoreactivity than by routine morphology. RET immunohistochemistry is a potentially useful new tool in the evaluation of patients for HD.

### 491 PML Protein Expression in Esophageal Carcinoma

*P Khanna, RA Delellis, YE Chin, LJ Wang.* Rhode Island Hospital and Brown University School of Medicine, Providence, RI.
**Background:** The product of the promyelocytic leukemia (PML) gene is a nuclear phosphoprotein that has been implicated in the inhibition of cell growth and transformation and in the induction of apoptosis. The PML gene was first identified at the non-random translocation breakpoint t(15:17)q(22,12) of acute promyelocytic leukemia (APL). Fusion of the PML gene with retinoic acid receptor alpha is thought to be a central mechanism in the development of APL. The PML protein (PMLP) is expressed in a variety of different normal and neoplastic cells and is considered a prime target for cancer prevention and treatment by retinoic acid analogues. However, the exact role of PMLP in tumorigenesis outside of APL is not clearly understood. There are no published data with regard to PML expression in esophageal tumors.
**Design:** Tissue microarrays were created from archival paraffin embedded tissue samples from 113 patients with distal esophageal lesions, of which 67 were adenocarcinoma, 26 were squamous cell carcinoma and 20 were benign lesions. In addition to tumor, cores of normal adjacent tissue were retrieved from the majority of cases. The microarrays were stained immunohistochemically with a mouse monoclonal antibody to PML gene product-1 (clone PG-M3). The results were recorded as 0 to 3+ based on the intensity of the stain.
**Results:** Moderate (2+) to weak (1+) nuclear staining was present in the basal layers of normal squamous epithelium. Benign lesions showed a staining pattern similar to that of normal mucosa. Among adenocarcinomas, 52 (78%) were negative, 2 (3%) were weakly positive (1+), 9 (13%) were moderately positive (2+) and 4 (6%) were strongly positive (3+). Among squamous cell carcinomas, 5 (19%) were negative and 21 (81%) were moderately to strongly (2-3+) positive. Of the poorly differentiated squamous cell carcinomas 69% were moderately (2+) to strongly (3+) positive.
**Conclusions:** There is a greater expression of PMLP in esophageal squamous cell carcinomas compared to adenocarcinomas ($p<0.0001$). In contrast to the previously studied loss of PMLP in many poorly differentiated carcinomas, high levels of PMLP expression are seen in predominantly poorly differentiated esophageal squamous cell carcinomas. Our results suggest that poorly differentiated esophageal squamous cell carcinomas may not respond to retinoid-based chemoprevention and therapy.

### 492 EphA2 Expression in Esophageal Cancers

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**Background:** The erythropoietin-producing hepatocellular (EPH) receptors represent the largest known family of receptor tyrosine kinases, which are activated by interaction with cell-surface ligands, termed ephrins. In normal cells, EphA2 expression appears to be restricted to intercellular junctions, where it binds to its ligands that are anchored to the membranes of adjacent cells. After binding, EphA2 becomes autophosphorylated resulting in a cascade of downstream signals that ultimately inhibit cell growth and migration. EphA2 is expressed at relatively low levels on a variety of adult epithelial cells and is widely upregulated in a variety of cancers of multiple histologic types. There are no published data comparing expression of EphA2 in different histologic subtypes of esophageal carcinomas.
**Design:** Tissue microarrays were created from archival paraffin embedded tissue samples from 113 patients with distal esophageal lesions, of which 67 were adenocarcinoma, 26 were squamous cell carcinoma and 20 were benign lesions. In addition to the tumor, cores of normal adjacent mucosa were retrieved from the majority of cases. The microarrays were stained immunohistochemically with mouse monoclonal antibody to EphA2, clone D7. The results were recorded as 0 to 3+ based on the intensity of the staining reaction.
**Results:** The normal squamous epithelium showed moderate cytoplasmic staining (2+) in the superficial layers in the majority of the cases. The benign lesions showed staining patterns similar to that of normal mucosa. Among adenocarcinomas, 62 (92.5%) were negative and 5 (7.5%) were weakly positive (1+). No significant relation was found between the EphA2 positivity and tumor differentiation in this group. Of 26 squamous cell carcinomas, 5 (19%) were negative and 21 (81%) were positive (2-3+). Among the positive cases, 95% showed stronger positivity in the well-differentiated squamous areas but did not show staining in the basal layer or in poorly differentiated areas. Three of 3 basaloid squamous cell carcinomas were negative.
**Conclusions:** There is a greater expression of EphA2 in esophageal squamous cell carcinomas compared to adenocarcinomas ($p<0.0001$). Well differentiated squamous cell carcinomas overexpress EphA2 (95%) and the expression is decreased in the poorly differentiated tumors.

### 493 Invasive Micropapillary Carcinoma (MPC) of Colon: An Aggressive Variant of Colonic Adenocarcinoma

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**Background:** Invasive MPC is an uncommon variant of carcinoma (Ca)s that is characterized by small papillary cell clusters surrounded by lacunar spaces and associated with a high incidence of lymph node (LN) metastasis. Although MPC has been described in other organs, such as breast, urinary bladder, lung, ovary, and salivary gland, it has never been described in colonic adenoCa. In this study, a clinicopathologic and immunohistochemical findings of MPC were compared with conventional adenoCa.
**Design:** 55 cases of colonic adenoCa with MPC component were identified among 585 consecutive cases of colon Ca at Asan Medical Center between Jan 2003 and June 2004. For comparison, 119 cases of conventional adenoCa of colon without MPC component were randomly selected and studied. Formalin-fixed, paraffin embedded tissue blocks were arrayed and immunostained for cytokeratin (CK) 7 and 20. We also compared the results of MLH-1, MSH-2, p53, and CEA immunostainings between MPC and conventional adenoCa.
**Results:** The patients' mean age of MPC was 62.6 years and that of conventional adenoCa was 58.8 years. Mean tumor size of MPC and conventional type was 5.6 cm and 5.8 cm, respectively. The grade of both MPC and conventional type was mostly moderately to poorly differentiated. The proportion of MPC ranged from 5% to 80%. MPC was more frequently associated with lymphovascular invasion (LVI), LN metastases, and greater mean numbers of positive LNs ($p<0.05$). However, the proportion of MPC component did not affect the biologic behavior. In 55 cases of MPC, 41 cases showed LN metastases with 15 cases containing MPC. In addition, MPC was

associated with a higher stage with frequent distant metastases than conventional adenoCa (p<0.001). CK7 staining was occasionally observed in both MPC (5 of 55 cases, 9.1%) and conventional adenoCa (16 of 119 cases, 13.4%) with slightly less CK7 expression in MPC group. Although MLH-1 expression tended to be lower in conventional adenoCa, the results for CK20, MLH-1, MSH-2, p53, and CEA were not statistically different between MPC and conventional adenoCa.
**Conclusions:** Recognition of MPC component is important since MPC appeared to be an aggressive variant of colonic adenoCa and presented at a higher stage with frequent LVI, LN metastasis, and distant metastasis than conventional adenoCa. The proportion of MPC component did not impact on the prognosis and the immunoprofiles of MPC were not significantly different from conventional adenoCa.

### 494 Additional Lymph Node Examination from Entire Submission of Residual Mesenteric Tissue (ESMT) in Colon Cancer Specimens May Not Add Clinical and Pathologic Relevance

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**Background:** Examination of lymph nodes (LN) is an important procedure, determining the tumor stage, adjuvant therapy, and prognosis of colorectal carcinoma. The number of metastatic LNs has been correlated significantly with the number of examined LNs. Although the current recommendation for N stage is to harvest at least 12 LNs in manual (standard) node dissection (MND), a recent study reported that ESMT might upstage about one-quarter of colorectal cancers from node negative to node positive. We analyzed the result of additional LN dissection by ESMT using fat clearing technique after MND.
**Design:** After MND of LN of 48 randomly selected colorectal carcinoma specimens from three separate institutions (UUH, AMC, NCC), the residual mesenteric tissue was entirely dehydrated for fat clearance over several days by daily washing in alcohol and acetone, and submitted for microscopic examination to evaluate the numbers of additional LNs, size of the LNs, and status of nodal metastasis.
**Results:** There were 29 pN0, 10 pN1, and 9 pN2 cases, respectively. Less than 12 LNs were harvested in 14 cases (29.2%) by MND. The mean number of LNs found by MND was 19.4 (range, 4-48 ; median, 19), while that of additional LNs from ESMT was 23.6 (range, 4-69 ; median, 22). The average number of additional blocks was 24.4 (range, 8-60 ; median, 20). The total 1,132 LNs were additionally found after ESMT. Most of the additional LNs (88.6%) were ≤ 2.0mm. Among them, 14 LNs (1.2%) in 10 cases revealed additional metastases. In all initial 29 pN0 cases, there was no additional nodal metastasis. In original 19 LN positive cases, there was additional node metastases in 10 cases (5 pN1 and 5 pN2 cases, respecively). Of these, two cases were upstaged from pN1 to pN2, which had <12 LNs in MND.
**Conclusions:** The MND seems sufficient for pN0 cases to be declared node negative irrespective of the original number of LNs. On the contrary, in LN positive cases, especially in pN1 with <12 LNs from MND, ESMT helped the correct pN status. However, in light of its minimal variation, clinical irrelevance in therapy decision, time consuming and cost, ESMT is not advised based on our results. MND seemed to be accurate and efficient for the evaluation of N stage in colorectal cancer specimens.

### 495 NFI Associated Gastrointestinal Stromal Tumors (GIST) Have Unique Clinical and Phenotypic and Genotypic Characteristics

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**Background:** The occasional occurrence of GIST in NF1 patients has been documented in several case reports and a few small series. This study aimed to evaluate the incidence of GIST among NF1 patients and to describe the phenotypic and genotypic characteristics of such tumors.
**Design:** 15 NF1 patients (8 males; 7 females; 19 - 81 yrs. old) with GIST were analyzed in terms of clinical presentation and course, morphology, and genetic rearrangements.
**Results:** 3 patients had a solitary GIST while 12 had multiple tumors (3 to >100) ranging in size from 1 mm. to 10 cm., involving the entire gastrointestinal tract. In 9 patients the tumors were detected due to symptoms; in the remaining 6 patients, they were incidental findings. The majority of tumors were of the spindle-cell type with a low mitotic rate. All but 2 of 50 immunostained GIST were strongly CD117 positive. CD117 immunostains also highligted diffuse and focal hyperplasia of the interstitial cells of Cajal (ICC). dHPLC analysis and DNA sequencing showed that none of 25 GIST had any KITor PDGFRα mutations.
**Conclusions:** NF1 is associated with a 100 - 200X increased risk of developing GIST. NF1 associated GIST are typically multicentric, of low malignant potential, and lack activating KIT or PDGFRα mutations, suggesting pathogenetic mechanisms different than those occuring in sporadic GIST.

### 496 Frequent Gastric Differentiation in Colorectal Carcinoma with Microsatellite Instability

*SF Kuan, AR Sepulveda.* University of Pittsburgh, Pittsburgh, PA.
**Background:** Serrated polyps often show microsatellite instability (MSI) and gastric differentiation. Polyps with this phenotype have an increased risk of progression to MSI-positive colorectal carcinoma (CRC), which are often histologically heterogeneous. CRCs may show heterogeneous expression of multiple genes, including MUC2 (intestinal goblet mucin), MUC5AC (gastric foveolar mucin), MUC6 (gastic pyloric mucin), cytokeratin 7 and 20, and CDX2 (a transcription factor that binds MUC2 promoter and activates MUC2). The aim of this study was to determine whether specific morphologic subtypes or molecular phenotypes of CRC correlate with tumor MSI status.
**Design:** Surgically resected CRCs which were selected after genotyping with a standard panel of microsatellite markers, include 20 microsatellite unstable (MSI) and 20 microsatellite stable (MSS) carcinomas. Immunostains for CDX2, MUC2, MUC5AC, MUC6, CK7 and CK20 were performed in representative tissue sections.
**Results:** Intestinal mucin MUC2 are equally well expressed in both MSI- and MSS-CRC and correlated with the expression of CDX2. MSI-CRC differs significantly from MSS-CRC in their expression of gatric mucins MUC5AC and MUC6 (p<0.00001 and p<0.003 respectively). Loss of CK20 and expression of CK7 is more common in MSI-CRC. Mucin expression was typically heterogeneous in tumors, being expressed in foci rather than a diffuse pattern. Five different morphologic patterns were identified in MSI-CRC: poorly differentiation(PD), tubular/villous (T/V), medullary (ME), mucinous (MC), and signet-ring cell (SRC). PD pattern is associated with global loss of antigens except for MUC5AC and MUC6. T/V and ME patterns are associated with strong expression of CDX2, MUC2, and CK20, but variable expression of MUC5AC and MUC6. MC and SRC patterns have broad expression of all 3 mucins.

IHC Reactivity

| Tumor type | CDX2(+) | MUC2(+) | MUC5AC(+) | MUC6(+) | CK7(+) | CK20(+) |
|---|---|---|---|---|---|---|
| MSI-CRC(N=20) | 15/20 | 15/20 | 18/20 | 14/20 | 5/20 | 13/20 |
| MSS-CRC(N=20) | 17/20 | 17/20 | 4/20 | 4/20 | 2/20 | 19/20 |

**Conclusions:** Colorectal cancers expressing gastric mucins MUC5AC and MUC6 in tumor foci are associated with microsatellite instability, suggesting these tumors may have developed from foci with gastric differentiation within serrated polyps. Identification of gastric mucin expression in tumors may be helpful to identify tumor foci for microsatellite instability analysis. Furthermore, MSI-CRC is a useful model for the study of tumor heterogeneity, and for the evaluation of molecular pathways of cancer progression from well-defined tumor foci.

### 497 Seasonal Pattern of Onset in Lymphocytic Colitis

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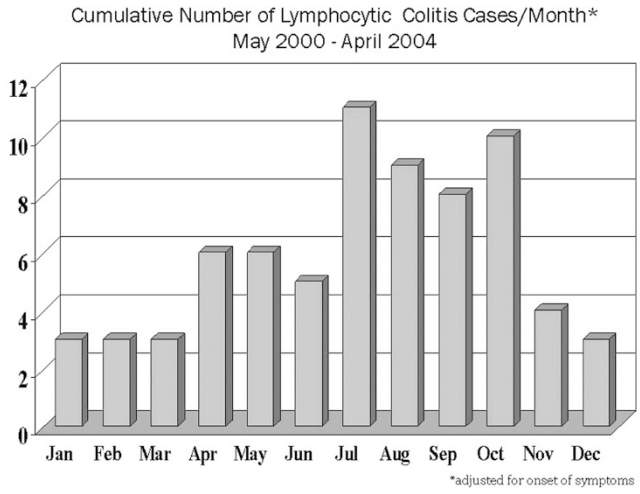
**Background:** The etiology of lymphocytic colitis (LC), a microscopic colitis syndrome, has remained elusive. Clinical and histologic similarities with collagenous colitis, celiac disease and certain medication-induced disease have all been reported. As in Brainerd diarrhea, a chronic colitis that may mimic LC microscopically, a link to some unidentified infectious source has also been suggested. Given that many infectious enteritides demonstrate seasonality, we chose to review cases of LC at our institution in order to determine if any variability in symptom onset existed.
**Design:** We undertook a retrospective review of LC cases diagnosed at our institution from May 2000 to April 2004. Cases were identified by a computerized search of the departmental database. Chart review was conducted to determine: (1) nature and duration of diarrhea, (2) month of onset of symptoms, (3) endoscopic findings, and (4) if evidence of celiac sprue or drug-induced colitis existed. Histologic inclusion criteria included: (1) diffuse colitis with intra-epithelial lymphocytes (IEL) counts > 10 lymphocytes/100 enterocytes, (2) evidence of surface epithelial damage, and (3) no significant neutrophil infiltrates, mucosal architectural distortion or subepithelial collagen deposits. Average IEL count was recorded for each case. Chi square test of homogeneity was applied to determine significance of perceived variability.
**Results:** Of the 71 cases meeting our criteria, M:F ratio was 1:2.2, median age at diagnosis was 59 years (range, 22-86), and median IEL count was 24 (range, 10-60). We found an excess number of cases during the summer/fall months and a paucity during winter months (Figure). The distribution of onset of symptoms was not appreciably different from year to year. Statistical analysis revealed a trend in seasonality when assesed at monthly intervals ($X^2$=15.9; p=0.14); whereas assessment at 2-month ($X^2$=14.5; p=0.013), 3-month ($X^2$=10.0; p=0.018), and 4-month ($X^2$=14.1; p=0.0008) intervals revealed statistically significant results.
**Conclusions:** Our observations of seasonal variability in the onset of LC in Vermont may support an association with an underlying infectious etiology.



### 498 Low-Grade Tubuloglandular Adenocarcinoma Associated with Inflammatory Bowel Disease: A Study of 21 Cases

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**Background:** Patients with extensive long-standing IBD face a substantial risk of developing colorectal adenocarcinoma. IBD-associated cancers are usually not morphologically distinctive, although mucinous and signet ring subtypes are disproportionately frequent. However, they include a group of low-grade tubuloglandular adenocarcinomas (LGTGA) that constitutes a distinct clinicopathological entity.

**Design:** Mount Sinai Hospital pathology database entries of 1993-2004 were searched for colectomy specimens with well-differentiated adenocarcinoma in patients with IBD. All available slides, specimen descriptions and clinical data were reviewed. Cases of LGTGA were identified based on a constellation of histological characteristics of either the entire tumor or at least its superficial (submucosal) components: very well-differentiated glands with circular, tubular or ameboid contours, cuboidal to low columnar lining epithelium with bland nuclear characteristics, absent or minimal intraluminal necrotic debris and absent or minimal stromal desmoplasia.

**Results:** Of 149 colectomy specimens with IBD-associated cancers, 17 (11%), comprising 12 ulcerative colitis, 3 Crohn's disease and 2 indeterminate colitis, contained a total of 21 cancers fulfilling the criteria for LGTGA. The mean age at surgery was 41.5y (range, 28-58). Five LGTGAs (24%) lacked any visible mucosal component and were discovered incidentally. The mucosa overlying all LGTGAs showed low-grade dysplasia alone (90%) or with focal high-grade dysplasia (10%) and appeared to give rise directly to the malignant glands. Eleven cancers (52%) were histologically uniform throughout, but 10 (48%) assumed more conventional, aggressive characteristics deep to the submucosa. The latter cancers tended to be of more advanced stages than the former and accounted for 2 instances (10%) of regional node metastasis. Of 13 cancers evaluated for cytokeratin expression, 9 (69%) expressed both CK7 and CK20. Of 11 cases evaluated for hMLH1 expression, silencing occurred in 6 (55%), suggesting deficient DNA mismatch repair.

**Conclusions:** LGTGA associated with IBD is a clinicopathological entity characterized by distinctive histology, frequently inconspicuous gross appearance, and direct derivation from mucosa with low-grade dysplasia. The risk of regional nodal spread is low unless the deep invasive component assumes more conventional histological features. High frequencies of CK7 expression and hMLH1 silencing suggest that LGTGA harbors unique molecular characteristics.

### 499 Mucosal Epithelioid Nerve Sheath Tumors; a Clinicopathologic Study of a Novel Mucosal-Based Neoplasm

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**Background:** Mucosal nerve sheath tumors have been well described in the gastrointestinal tract and other mucosal sites. In a series of mucosal biopsies, we have encountered a distinct subset of mucosal peripheral nerve sheath tumors characterized by small epithelioid cells and a benign clinical course.

**Design:** A series of 6 mucosal biopsies [5 colonic, 1 bladder] was received by a single large institution in consultation. The histological and clinciopathologic features of the cases were reviewed.

**Results:** The mean age at presentation was 58.7 years with a female predominance (4 F, 2 M). Four of the colonic lesions were from the left colon and one from the right colon. The bladder biopsy was from the bladder neck. All of the colonic lesions were discovered as small [0.2 to1.0 cm] polyps during the time of colonoscopy [3 at the time of routine screening, 2 for the workup of occult blood in the stool]**.** The bladder neck mass was seen on bladder ultrasound after the patient presented with vaginal bleeding**.** None of the patients had a known history of neurofibromatosis.

Histologically, the lesions showed an infiltrative growth pattern and were composed of spindled to predominantly epithelioid cells arranged in nests and whorls. The epicenters of the lesions were located in the lamina propria and extended to the superficial submucosa. The proliferating cells had uniform round to oval nuclei with frequent intranuclear pseudoinclusions and eosinophilic fibrillary cytoplasm. No mitoses were seen. All lesions expressed diffuse S100 staining and 4 of 5 lesions stained showed CD34 positivity in supporting cells. All were negative for CD117. One showed superficial mucosal erosion, and one had an inflammatory infiltrate predominantly composed of eosinophils.

In follow up of five patients, none have had any symptoms or recurrence of disease.

**Conclusions:** Mucosal epithelioid nerve sheath tumors are a rare novel entity characterized by prominent epithelioid round to oval cells with an infiltrative growth pattern. These lesions are often discovered incidentally and have a benign clinical course.

### 500 Enterocolic Lymphocytic Phlebitis in Patients with Collagenous and Lymphocytic Colitis

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**Background:** Enterocolic lymphocytic phlebitis (ELP) is a rare form of intestinal vasculitis defined by lymphocytic +/- granulomatous inflammation that is restricted to mural and mesenteric veins and spares the arterial system and lymphatics. It is thought that superimposed thrombosis in ELP impairs blood flow away from the bowel with secondary ischemia and necrosis. ELP has to date been described almost exclusively in cases of ischemic bowel disease, including one recent report of ELP in a patient with ischemia and a background of lymphocytic colitis. There have been no series evaluating the prevalence of ELP in microscopic colitis (lymphocytic or collagenous colitis) without ischemia.

**Design:** An index case was identified in which a 70-year-old woman underwent total colectomy for intractable diarrhea associated with pseudomembranous collagenous colitis. The colectomy specimen demonstrated extensive and well-developed ELP in addtition to collagenous colitis, but (unlike prior reported cases of ELP) there was no ischemic injury. To define the scope of ELP in patients with microscopic colitis, we evaluated 5 additional partial or total colectomy specimens containing collagenous (CC) or lymphocytic colitis (LC).

**Results:** Of these 5 additional colectomies, 4 were in patients with CC (2 segmental resections performed for colonic adenocarcinoma and 2 total colectomies for intractable diarrhea) and 1 total colectomy for diarrhea in a patient with LC. Two total colectomy sepcimens (1 CC and 1 LC) showed transmural perivenular lymphocytic inflammation, but in both cases the infiltrates were quite mild and patchy and did not reach our diagnostic threshold for ELP. Both segmental colectomies for colonic adenocarcinoma revealed transmural perivenular inflammation, but unlike ELP the infiltrates were restricted to the bowel wall subjacent to the carcinoma and also surrounded lymphatic spaces.

**Conclusions:** The index case we present is the first example of ELP associated with collagenous colitis and the first report of ELP in a non-ischemic bowel. While the index case is the only unequivocal instance of ELP in our series of 6 microscopic colitis (16%), focal venulitis was also present in 2 other total colectomy specimens showing microscopic colitis (33%). The finding of ELP in a patient suffering from severe collagenous colitis suggests that in some cases ELP may be a reactive, secondary lesion to an altered mucosal barrier, rather than a specific cause of ischemic bowel disease.

### 501 Significance of the Depth of Tumor Invasion in Early (T1) Esophageal Adenocarcinoma

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**Background:** The patients with early (T1) esophageal adenocarcinomas have an excellent prognosis after esophagectomy. These early stage T1 adenocarcinomas are composed of a heterogeneous group of tumors including tumors invading into mucosa and submucosa. It is unclear if the depth of tumor invasion can predict the clinical outcome. In this study, we evaluated the association between depth of tumor invasion, lymph node metastasis, survival and recurrence rate in early stage esophageal adenocarcinomas.

**Design:** 76 consecutive patients with resected early (T1) adenocarcinoma of esophagus from MD Anderson Cancer Center were evaluated. The T1 tumor were classified into four groups based on the depth of tumor invasion: T1a, invading into lamina propria; T1b, invading into muscularis mucosa, T1c, invading into superficial (<50% of thickness of submucosa) submucosa; and T1d, invading into deep (≥50% of thickness of submucosa) submucosa. The depth of tumor invasion was evaluated with lymph node metastasis, survival rate, and disease recurrent rate.

**Results:** Barrett's mucosa was present in 92% (70 of 76) of the resected specimens. Lymph node metastasis was more frequent in tumors invading into submucosa (26%; 8 of 31) than in tumors confined to mucosa (4%, 0/34 in T1a and 2/11 in T1b, p=0.01). In tumors invading into submucosa, lymph node metastasis was significantly associated with depth of tumor invasion, 40% (8 of 20) in T1d and 0% (0 of 11) in T1c (p=0.02). Tumor recurrence was more frequent in tumors invading into submucosa (45% at 5 years) than in tumors confined to mucosa (0% at 5 years, p<0.0001). However, there was no difference in overall survival and disease free survival between tumors invading into submucosa and mucosa, and between tumors with or without lymph node metastasis.

**Conclusions:** Tumor invasion into submucosa was significantly associated with lymph node metastasis and high tumor recurrence rate in patients with T1 esophageal adenocarcinomas, but was not associated with patients' survival.

### 502 CpG Island Methylation in Carcinoid Tumors and Pancreatic Endocrine Tumors

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**Background:** Carcinoid tumors and pancreatic endocrine tumors (PETs) are uncommon neuroendocrine neoplasms, and their genetic and epigenetic alterations are not well characterized. These tumors have site-specific differences in neuroendocrine characteristics, clinical course and genetic alterations.

**Design:** We evaluated the methylation profiles of the *p14*, *p16*, and *O⁶-methyl-guanine methyltransferase* (*MGMT*) genes in 47 patients with neuroendocrine tumors including 17 with PETs, 14 with non-ileal carcinoid tumors and 16 with ileal carcinoid tumors by bisulfite treatment of DNA and methylation specific-polymerase chain reaction. The methylation profile was correlated with clinicopathologic features.

**Results:** As compared to patients with PETs patients with carcinoid tumors had more frequent history of alcohol consumption (p=0.03), and ileal carcinoid tumors more frequently had lymph node (p=0.02) and liver metastasis (p=0.02). Methylation of the *p14* gene was present in 49% (23/47) of tumors, *p16* gene in 26% (12/47) of tumors, and *MGMT* gene in 13% (6/47) of tumors. Methylation of the *p14* gene was present in 41% of PETs, 36% of non-ileal carcinoid tumors and 69% of ileal carcinoid tumors; of *p16* gene in 18%, 36% and 25% of tumors; and of *MGMT* gene in 12%, 29% and 0% of tumors (p=0.03), respectively. Methylation of *p16* gene was associated with older age of patients (p=0.002) and liver metastasis (p= 0.04).

**Conclusions:** Our study indicates that methylation of *p14, p16 and MGMT* genes frequently occur in carcinoid tumors and PET. The methylation profiles vary by tumor subsite and associated with clinicopathologic features.

### 503 Alterations of Mucosal Cell Populations in the Pathogenesis of Irritable Pouch Syndrome

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**Background:** Irritable pouch syndrome (IPS) is a recently described entity in patients with ileal pouch-anal anastomosis (IPAA). IPS is characterized by diarrhea, urgency, and abdominal pain while lacking evidence of inflammation of the pouch on endoscopy and histology. IPS bears clinical features of irritable bowel syndrome (IBS). The etiology and pathogenesis of IPS are not known. Our recent study using electronic barostat revealed evidence of visceral hypersensitivity in patients with IPS, the same mechanistic features seen in IBS. We hypothesize that factors implicated in the etiology of IBS including alteration in lymphocytes, mast cells, and serotonin-containing enterochromaffin (EC) cells may also contribute to the development of IPS. This study was designed to evaluate these cells in pouch mucosa in patients with IPS.

**Design:** Symptoms, endoscopy, and histology were assessed in 19 patients having IPAA for medically refractory ulcerative colitis (UC) or UC with dysplasia. IPS (n=10) was defined as the presence of diarrhea, abdominal pain, and pelvic discomfort in the absence of inflammation of the pouch and rectal columnar cuff on endoscopy and histology. Asymptomatic patients (n=9) with no inflammation on pouch endoscopy and histology served as the controls. A gastrointestinal pathologist, blinded to demographic, clinical, and endoscopic information, assessed and scored the following variable: neutrophilic inflammation, intraepithelial lymphocytes, lamina propria CD3+T cells, tryptase and CD25 immunostains for mast cells, and serotonin immunostain for EC cells.

**Results:** Both groups were compatible demographically in terms of age, gender, duration of UC, stage, indication, and duration of the pouch surgery. There was no difference in degree of acute inflammation, and numbers of intraepithelial lymphocytes, CD3+ T cells, and mast cells. The group with IPS showed a mean count of 52.2±29.8 EC cells per ten 400x fields, while the control group had 32.7±16.6 EC cells ($P$=0.19). This showed a trend of elevated EC cells in patients with IPS.

**Conclusions:** This is the first study of its kind to investigate the cellular mechanism of IPS. This pilot data suggest that an increased number of serotonin-expressing EC cell population in pouch mucosa may provide a molecular basis for visceral hypersensitivity in patients with IPS. In contrast, inflammatory cellular components, including lymphocytes and mast cells, may play a limited role in the pathogenesis of IPS.

### 504 A Novel Set of Suppressor Genes (RSK4, KIA0828, tip60) Are Highly Downregulated in Colon and Lung Carcinomas

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**Background:** Malignant transformation requires accumulation of multiple genetic alterations related with cell signaling, cell cycle, apoptosis, cell invasiveness,.. Importantly, this myriad of genetic events use to occur in immortalized cells. Recently, in a large-scale RNA interference screen, five genes (RSK4, HDAC4, KIAA0828, TCP1 and tip60) which modulate p53-dependent proliferation arrest were identified.

**Design:** 20 colon carcinomas, 20 lung carcinomas and 20 prostate carcinomas were analyzed. RNA was extracted from both normal and tumor tissues of each patient. The real-time PCR was performed using Taqman probes corresponding to RSK4, HDAC4, KIAA0828, TCP1, tip60 and p53 genes (ABI PRISM 700 SDS). As an endogenous control gene, ciclofilin was amplified. Positive controls for each gene were also included.

**Results:** In colon carcinomas, the genes RSK4, HDAC4, KIAA0828 and tip60 were downregulated. RSK4 and KIAA0828 showed a striking decrease of mRNA level in all the cases (p<0,001). In lung carcinomas, HDAC4, KIAA0820 and tip60 were also down regulated (p<0,01) and in prostate cancer only a light downregulation of gene KIAA0828 was observed.

**Conclusions:** A striking downregulation of potential new suppressor genes RSK4, KIAA0828 and tip60 have been detected in colon carcinomas and in a subset of lung carcinomas. Expression of these genes that may control p53 function as well as ras-MAPK pathways and methylation and transcriptional cellular programs, can represent a novel set of regulatory suppressor genes involved in human tumors.

### 505 Clinicopathological Significance of the c-KIT and PDGFRα Gene Mutations in Patients with Localized Gastrointestinal Stromal Tumors: A Study of the Spanish Research Sarcoma Group (GEIS)

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**Background:** The purpose of the present study is to analyze the prognostic relevance for disease-free survival (DFS) of clinicopathological, immunophenotypical and mutational variables in complete surgical resected GIST patients.

**Design:** Three hundred and fifty patients with GIST from 29 Spanish hospitals were evaluated. One hundred and seventy-one patients were selected for the molecular and prognostic analysis in accordance with the following criteria: complete surgical resection; c-kit +; no metastasis at presentation; tumor size grater than 2 cm; and absence of second primary tumor. Mutational analysis of the c-KIT (exons 11, 13 and 17) and PDGFR (exons 12 and 18) genes and PDGFRa immunostaining were also analyzed.

**Results:** Thirty out of 171 (18%) cases were high grade tumors . One hundred and thirty-one (77%) were spindle tumors, the sarcomatoid variant being higher in the high grade tumors (p<0.001). c-KIT expression was higher in spindle than epithelioid tumors (p<0.001), whereas 67% of epithelioid tumors expressed PDGFRα. We found 97 mutations (57%): 83 in the c-KIT gene (49%) and 14 in the PDGFRa (8%). KIT mutations were associated with KIT expression (p=0.02) and inversely correlated with PDGFRa immunostaining (p=0.06). KIT mutations were more frequent in spindle tumors. In this series, mitotic count (p=0.00001); the hypercellularity (p=0.00001), tumor size (p=0.0009), gender (p=0.0136), and location of the tumor (p=0.019) presented the worst prognosis. After the multivariate analysis, tumor size, mitotic count and cellularity had an independent prognostic value. Positive immunostaining of the PDGFRα (p=0.007), as well as the exon 18 PDGFRα mutations (p=0.1174) showed a favourable DFS, whereas deletion type and missense mutations in exon 11 of c-KIT showed a poor and favorable outcome respectively.

**Conclusions:** High cellularity and mitotic count and size are the major prognostic factors for the DFS. PDGFRα negative immunostaining discriminate a poor subgroup in GIST patients. Further analysis may demonstrate the prognostic and predictive value of the type of mutation in the c-KIT and PDGFRα genes.

### 506 Basal Crypt Dysplasia-Like Atypia with Surface Maturation in Barrett's Esophagus: Evidence That It Represents True Neoplasia

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**Background:** Rarely, we have noted that mucosal biopsies from Barrett's esophagus (BE) may show dysplasia-like atypia in the bases of the crypts but without involvement of the surface epithelium (i.e. surface maturation is present) (BCDSM). However, it is commonly believed that lack of surface maturation is a necessary feature to establish a diagnosis of true dysplasia. The aim of this study was to evaluate the clinical, pathologic, molecular and immunohistochemical characteristics of 15 BE patients who have BCDSM in their surveillance endoscopic mucosal biopsies in order to gain insight into its biological significance.

**Design:** Routinely processed mucosal biopsies from 15 BE patients with BCDSM were identified from a cohort of 201 consecutive BE patients undergoing endoscopic surveillance for BE (prevalence; 7.5%), and evaluated for several clinical, pathologic, immunohistochemical (p53 and MIB-1 by the ABC method) and molecular markers (elevated 4N fraction, aneuploidy).

**Results:** All BE patients (M/F ratio; 12/3, mean age; 73 yrs, mean length of BE; 6.2 cm, mean duration of BE; 94 mths), except two, had dysplasia detected in biopsies either prior to, or concurrent with, the one that contained BCDSM. In fact, two patients had adenocarcinoma present in biopsies elsewhere in the BE. Compared to adjacent non-atypical and non-dysplastic (metaplastic) epithelium, areas of BCDSM showed p53 positivity in 9/15 cases (9/15 vs. 0/15; p<0.001) and a significantly elevated MIB-1 proliferative rate (0.46±0.03 vs. 0.27±0.04, p<0.001). Compared to known dysplastic epithelium, areas of BCDSM showed a similar degree of p53 positivity, and MIB-1 proliferation rate (0.46±0.03 vs. 0.37±0.05, p>0.2). 10/15 (67%) and 7/15 (47%) patients showed elevated 4N fraction and aneuploidy, respectively.

**Conclusions:** Basal crypt dysplasia-like atypia with surface maturation in mucosal biopsies from patients with BE is an uncommon, but probably significant, pathologic change that shows a high association with dysplasia and/or carcinoma. As such, this morphologic alteration may, in fact, represent a subtype of true dysplasia despite the morphologic characteristic of apparent surface maturation.

### 507 Aberrant Expression of Maspin in Idiopathic Inflammatory Bowel Disease Is Associated with Disease Activity and Neoplastic Transformation

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**Background:** Maspin (SERPINB5), a serine proteinase inhibitor, is overexpressed in inflammatory states as well as in many cancers, supporting a potential role in inflammation-associated neoplasia. Idiopathic inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease) is associated with an increased risk for developing colorectal dysplasia and cancer. Whether maspin contributes to disease activity and neoplastic transformation in IBD has not been examined.

**Design:** Tissue microarrays (TMAs) were generated from archival tissue from 125 patients with IBD and/or IBD-associated dysplasia or cancer, including 30 with inactive chronic disease, 51 with active chronic disease, 4 with colitis-associated epithelial changes indefinite for dysplasia (IFD), 7 with colitis-associated low grade dysplasia (LGD), 8 with colitis-associated high grade dysplasia (HGD) and 25 with colitis-associated invasive adenocarcinoma; in addition, 9 normal colonic mucosal specimens from non-IBD patients were also arrayed. Each case was represented by up to 8 cores. Immunolabeling was scored as negative (expression in < 5% lesional cells), focal (5-25% expression) and diffusely positive (>25% expression); only epithelial labeling was scored.

**Results:** Focal maspin expression was present in only 1/9 (11%) normal colonic samples. In contrast, maspin was expressed in 47/51 (92%) active IBD samples, which was significantly higher than both inactive IBD (13/30, 43%) and normal mucosa (P < 0.01); in particular, the diffuse pattern of maspin expression was significantly higher in active IBD (41/51, 80%), compared to inactive IBD (5/30, 17%) and normal mucosa (0%) (P < 0.01). In the multistage progression model of colitis-associated neoplasia, aberrant maspin labeling was observed at the earliest stages, with 3/4 (75%) IFD, 6/7 (86%) LGD, and 8/8 (100%) HGD specimens expressing maspin, virtually always in the diffuse pattern. Expectedly, 22/25 (88%) of invasive IBD-associated cancers overexpressed maspin, including 21 with diffuse labeling.

**Conclusions:** Maspin is significantly overexpressed in both active IBD and colitis-associated dysplasia than either inactive IBD or normal colonic mucosa, suggesting a potential role in disease "flare" as well as neoplastic progression. Targeting maspin for control of disease activity and cancer prophylaxis may be a promising novel therapeutic strategy for IBD.

### 508 What Is the Value of Endoscopic Terminal Ileal Biopsies?

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**Background:** Biopsies of the terminal ileum (TI) are common specimens, often taken during colonoscopic exams for a variety of indications, including unexplained diarrhea, pain, suspected or established inflammatory bowel disease and abnormal imaging studies. Little data exists on the diagnostic value of such biopsies.

**Design:** 415 consecutive TI biopsies were studied. Hematoxylin and eosion-stained slides were reviewed for significant inflammatory changes, including distortion, ulcers, plasmacytosis, and pyloric gland metaplasia. The number of biopsies was recorded. The histologic findings were compared with the endoscopic appearances, as recorded on the endoscopic reports, and the number of biopsies.

**Results:** In 318 cases the TI was normal by endoscopy, and in 96% of these cases the biopsies were also normal. In contrast, in the 97 cases in which the TI was endoscopically abnormal, 63% had significant histologic inflammation. The yield was highest when the endoscopic features were ulcers, erosions and ileitis (81%) and lowest for endoscopic nodularity (25%). Statistically significantly more patients with endoscopic and histologic abnormalities had four or more biopsies compared to those with normal endoscopy and/or histology.

**Conclusions:** Overall the yield of significant histologic inflammation in biopsies of endoscopically normal TI is so low that biopsies in this situation are probably not helpful enough to be warranted. In contrast, when the TI has significant endoscopic inflammation, ulcers or erosions, biopsies of those abnormalities are informative more than 80% of the time, and are warranted. Endoscopists sample endoscopically abnormal TI more than they sample normal mucosa

### 509 Interobserver Agreement in the Evaluation of Pre-Resection Biopsies with at Least High Grade Dysplasia (HGD) in 163 Barrett's Esophagus (BE) Patients

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**Background:** Studies have documented poor interobserver agreement when interpreting the changes at the lower end of the metaplasia-dysplasia spectrum in biopsies from BE patients (negative v. indefinite v. low-grade dysplasia). However, no published data exist concerning the same issue in biopsies with changes at the upper end of the spectrum (HGD v. intramucosal (IMC) v. submucosal (SMC) adenocarcinoma). Since treatment options for patients with HGD/IMC/SMC now may include ablative therapies and endoscopic mucosal resection as alternatives to esophagectomy, accurately distinguishing these diagnostic categories in pre-treatment biopsies has become more critical since some treatments depend upon a diagnosis of "cancer". Moreover, recent studies have suggested a role for endoscopic surveillance for HGD, reserving esophagectomy for those with "cancer".

**Design:** Pre-resection biopsies from 168 BE patients with at least HGD who ultimately underwent esophagectomy were reviewed. A single slide showing the most severe abnormalities was selected from each patient by two pathologists. At a multiheaded microscope, all study pathologists evaluated five slides and developed a consensus set of eight histologic criteria defining four diagnostic categories: 1-HGD; 2-HGD with marked glandular architectural distortion, cannot exclude IMC; 3-IMC; 4-SMC. The remaining 163 slides were independently reviewed by six pathologists utilizing these criteria. Reviewers also recorded specific histologic criteria used to categorize each case. Kappa statistics were calculated to assess interobserver agreement.

**Results:** Although in 73.6% of cases at least 4/6 pathologists agreed (4/6: 36.8%, 5/6: 27.6%, 6/6: 9.2%), using kappa statistics, which account for agreement occurring by chance alone, the overall agreement was poor (k=0.30). Agreement for HGD (category 1) was moderate (k=0.46), but agreement for the other three categories (categories 2-4) was poor (k= 0.21, 0.31 and 0.14, respectively). Histologic criteria utilized to categorize each case varied, even among 13 IMC cases with perfect agreement.

**Conclusions:** These results call into question treatment regimens which are based on the assumption that HGD, IMC, and SMC can reliably be distinguished in endoscopic biopsies.

### 510 Microsatellite Stable and Unstable Mucinous Colorectal Adenocarcinomas Show Similar Expression Profiles for MUC1, MUC2, MUC4 and MUC5AC but Differ in MUC6

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**Background:** Previous studies have shown that microsatellite unstable (MSI) colorectal cancer (CRC) show a distinct clinicopathologic profile, including better prognosis, right sided location, and mucinous morphology. Microsatellite stable (MSS) and unstable CRCs have been demonstrated to show different mucin expression profiles. We directly compared mucinous CRC from these groups to determine whether differences in mucin expression are due to mucinous histology rather than MSI status.

**Design:** Nine MSI (MSI-high) and 14 MSS mucinous CRC were consecutively retrieved from archival files and diagnoses were confirmed. Greater than 50% mucinous features were necessary for the diagnosis of mucinous adenocarcinoma. Tumors had been previously evaluated for MSI using a modified Bethesda panel of microsatellite markers. Formalin-fixed paraffin-embedded sections were cut and stained using monoclonal antibodies against MUC1, MUC2, MUC4, MUC5AC, and MUC6. Staining was interpreted as positive if greater than or equal to 5% of cells showed staining and controls stained appropriately.

**Results:** Of the 9 MSI CRC; 1 (11%) stained for MUC1, 9 (100%) were positive for MUC2 and MUC4, 3 (30%) were positive for MUC5AC, and 3 (30%) were positive for MUC6. Of the 14 MSS CRC; 1 (7%) was positive for MUC1, 9 (100%) were positive for MUC2, 11 (78%) were positive for MUC4, 2 (14%) were positive for MUC5AC, and none was positive for MUC6. The only significant difference in mucin expression between groups was in MUC6 expression (p=0.04). Seven of the MSS cases were right sided, six left sided, and one transverse colon; while 5 of MSI cases were right sided, one left sided, one transverse colon, and 2 multifocal. The average patient age was 65.1 years old in patients with MSI tumors and 63.5 in those with MSS tumors.

**Conclusions:** It has been well demonstrated that MSI CRC are more frequently mucinous than MSS CRC. When only mucinous carcinomas are evaluated, as in this study, most of the reported differences in the mucin expression profiles between MSI and MSS carcinomas are no longer identified suggesting that the reported differences are likely due to differences in tumor type and differentiation rather than the MSI-status. MUC6 expression was the only mucin that was differentially expressed in the MSI and MSS mucinous CRC and was only expressed in MSI tumors. MUC6 is classified as a secretory gel forming mucin, and the clinical significance of its expression in MSI CRCs is not known.

### 511 Management of Superficial Barrett's Epithelium (BE) Related Neoplasms by Endoscopic Mucosal Resection (EMR): Evaluation of 27 Cases

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**Background:** EMR is being advocated for the treatment of BE related superficial neoplasms and some indicate that it represents a valid alternative to esophagectomy with low morbidity and recurrence rate. However, recent studies revise downward its success rate particularly regarding the ability to achieve complete excision, and instead advocate EMR as a diagnostic and staging tool. We herein report our experience with a series of EMRs, emphasizing its diagnostic, staging and therapeutic aspects.

**Design:** We evaluated the clinicopathological features of 27 esophageal EMRs (20 lesions in 18 patients) and correlated the initial biopsy and pre-EMR endoscopic ultrasound (EUS) staging with the final histologic diagnosis and stage. Recurrence was correlated with EMR margin status.

**Results:** The neoplasms consisted of 2 low-grade dysplasias (LGDs), 8 high-grade dysplasias (HGDs), 14 intramucosal carcinomas (IMCs) and 3 submucosal invasive carcinomas (Inv CAs). The mean size of the lesions was 11 mm. EUS staging correctly reported an intramucosal or submucosal lesion in 70% of the cases while it over-staged 18% and under-staged 12% of the cases. The biopsy diagnosis corresponded to the EMR diagnosis in 68% of the cases. The biopsy under-estimated the grade of the lesion in 18% of the cases. 3 HGDs on biopsy were diagnosed as IMC and 1 IMC was upgraded to Inv CA on EMR. The EMR showed a lower histologic grade compared to the biopsy in 14% of the cases. 2 HGDs on biopsy were graded as LGD on EMR. One Inv CA on biopsy was graded as IMC on EMR. The resection was microscopically complete in only 4% of the cases. No residual / recurrent disease was observed in 10 lesions (9 patients) at 4-63 months (mean: 23 mo.) post EMR. However, 9 lesions (8 patients) including one resected 6 times persisted / recurred 28 days to 25 months (mean: 6 mo.) after treatment. 56% of the cases with positive lateral margin(s) and negative deep margin persisted / recurred. However, 86% of the EMRs with positive deep margin showed residual tumor / recurrence on follow up biopsies. Photodynamic therapy was performed after EMR in 14 lesions but it had no effect on persistence / recurrence.

**Conclusions:** EMR offers improved diagnosis and staging as compared to biopsy and EUS. This is a significant advantage since it can modify patients' management. However, frequent incompleteness of resection and high persistence / recurrence are significant pitfalls that dictate continued endoscopic surveillance.

### 512 Loss of Cables, a Cyclin-Dependent Kinase (CDK) Interacting Protein, Is Frequent in Intestinal Type Gastric Dysplasia and Early Gastric Cancer

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**Background:** Cables, a novel tumor suppressor gene which maps to chromosome 18q, interacts with multiple cyclin dependent kinases (cdks) and links the cdks and non-receptor tyrosine kinases. A previous study demonstrated loss of Cables expression in 40% of colonic adenomas and 65% of colorectal adenocarcinomas. Loss of heterozygosity at chromosome 18q is common in gastric cancers, raising the possibility of a tumor suppressor gene on chromosome 18q. The aim of this study is to analyze the possible role of Cables in the gastric carcinogenesis in its early stage.

**Design:** Our study group consisted of 26 cases of resected gastric dysplasia and early carcinoma, including low grade dysplsia (LGD) (n=3), high grade dysplasia (HGD) (n=1), intramucosal carcinoma (IMC) (n=17) and submucosal invasive carcinoma (Inv CA) (n=5). The immunohistochemical study using an affinity purified polyclonal antibody against Cables in each case. Nuclear staining of Cables was graded as positive (no loss), partial loss and complete loss of expression and correlated with neoplastic grade and histologic type.

**Results:** Twenty tubular type neoplasms (3 LGDs, 1 HGD, 14 IMCs and 2 Inv CAs), 3 papillary adenocarcinomas (2 IMCs and 1 Inv CA) and 3 signet ring cell carcinomas (2 IMCs and 1 Inv CA) were identified. Benign gastric epithelium including intestinal metaplasia showed preserved nuclear staining of Cables except for proliferative basal region of the glands. Complete loss of Cables nuclear staining was noted in 7 cases (1 LGD, 4 IMCs and 2 Inv CAs), partial loss in 6 cases (2 LGDs and 4 IMCs) and no loss in 13 cases (1 HGD, 9 IMCs and 3 Inv CAs). There was no correlation between Cables expression and neoplastic grade. All (3) cases of signet ring cell

carcinoma showed preserved Cables expression, while loss of Cables expression was seen in 55 % (11) of tubular type gastric neoplasms (6 complete; 5 partial) and 67 % (2) of papillary adenocarcinoma (1 complete; 1 partial).
**Conclusions:** Complete or partial loss of Cables expression is frequently seen in intestinal type gastric dysplasia and early gastric cancers. Our results indicate that inactivation of Cables may play a role in the pathogenesis of intestinal type gastric cancers.

### 513 Protein Kinase C theta (PKC-θ) Selective Expression in Gastrointestinal Stromal Tumors (GISTs)

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**Background:** PKC-θ is a serine/threonine protein kinase of the $Ca^{2+}$ independent novel PKC subfamily, usually expressed in lymphoid tissue, particularly in T-cell lymphocytes and T-cell neoplasias, certain parts of the nervous system and skeletal muscle. PKC-θ expression has been recently shown in interstitial cells of Cajal (ICC) and in GISTs, a tumor possibly originated in these cells. In a recent study (*Clin Cancer Res 2004;10:4089-4095*) we have demonstrated the selective expression of PKC-θ in a series of GISTs compared to other mesenchymal and epithelial tumors.
**Design:** The aim of the current study was to confirm the specificity of PKC-θ expression in a larger series of GISTs and GISTs metastasis and to determine the possible relationship between the expression and different clinicopathologic characteristics of the tumors, such as risk of aggressive behavior and the histologic pattern. We also evaluated the PKC-θ expression in some gastrointestinal mesenchymal tumors that present difficulties in the differential diagnosis with GISTs.
**Results:** A total of 90 GISTs and 18 gastrointestinal mesenchymal lesions, including 5 GIST-mimicking lesions with positive cytoplasmic KIT expression, were tested. Expression of PKC-θ was observed in all GISTs, whereas none of the gastrointestinal non-GIST mesenchymal tumors showed PKC-θ positivity. This series included only one case focally positive for KIT that was also positive for PKC-θ. No statistically significant differences were found regarding of intensity of expression and the risk of aggressive behavior or the histologic pattern. Only in 4 cases of malignant epitelioid tumors and their metastasis the pattern of PKC-θ expression was different, with a weak diffuse positivity of the tumor with some isolated or small groups of more atypical cells showing a moderate or intense positivity. The five lesions with cytoplasmic KIT expression were also negative.
**Conclusions:** In conclusion, PKC-θ immunohistochemical expression is a very sensitive and specific marker that may be of the most relevant interest for the histologic diagnosis of GIST, specially in cases were KIT expression is very weak or negative and in the differential diagnosis between GIST and GIST-mimicking lesions, specially using paraffin-embedded tissues.

### 514 Identification of Novel Cellular Targets in the Progression Model of Gastric Adenocarcinoma

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**Background:** About 20,000 new gastric cancers are diagnosed annually in the US and ~ 12,000 patients die of disease. Better understanding of gastric carcinogenesis should result in directed diagnostic and therapeutic targets.
**Design:** The publicly available Serial Analysis of Gene Expression (SAGE) database (http://www.ncbi.nlm.nih.gov/SAGE/) was used to query libraries of gastric cancer and normal stomach to identify differentially overexpressed genes in gastric carcinomas. A subset of 6 upregulated transcripts was subsequently validated at the protein level by immunohistochemical labeling of tissue micro arrays (TMAs), constructed from 133 gastric adenocarcinoma resection specimens, with known patient outcome. TMAs reflected the multistep model of gastric carcinogenesis; cores from normal gastric mucosa, intestinal metaplasia (IM, when present), primary invasive cancer, and metastases (when present) were arrayed from a single case.
**Results:** Of the 6 proteins examined, expression in normal gastric mucosa varied from 0% (mesothelin) to 9% (TOPOII). One antigen (stratifin) was aberrantly expressed in 97% of IM, while the remaining were minimally expressed or absent. All but c-erbB-2 demonstrated significantly higher expression in invasive cancer and in metastases compared to nonneoplastic gastric mucosa ($P<0.05$), validating the findings of SAGE analysis. Expression of these proteins did not correlate with patient outcome.
**Conclusions:** Stratifin is upregulated early in the multistep progression of gastric cancer (at the IM stage), whereas c-erbB-2, fascin, mesothelin, S100A4, and TOPOII upregulation are later events. Incorporation of these findings would help design rational early detection and treatment panels.

Summary of Expressed Proteins and Outcome

| Protein | Fraction, % Staining, Stomach | IM | Primary Ca | Metastatic Ca | Associated with Outcome? |
|---|---|---|---|---|---|
| Stratifin | 2/66, 3% | 29/30, 97% | 111/120, 93% | 69/71, 97% | No |
| Fascin | 4/105, 4% | 1/7, 15% | 81/120, 67% | 51/70, 73% | No |
| Mesothelin | 0/98, 0% | 0/16, 0% | 79/119, 66% | 42/70, 60% | No |
| S100A4 | 2/107, 2% | 0/7, 0% | 90/117, 77% | 59/70, 84% | No |
| TOPOII | 10/108, 9% | 3/17, 18% | 75/124, 60% | 44/74, 59% | No |
| Erb-2 | 1/107, 1% | 0/15, 0% | 14/126, 11% | 11/77, 14% | No |

### 515 Morphology and Clinical Outcome of Residual Esophageal Carcinoma Following Neoadjuvant Therapy

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**Background:** The survival advantage of neoadjuvant therapy prior to resection for patients with esophageal cancer is established. While two studies have described the significance of persistent acellular mucin pools and neuroendocrine cells in esophagectomy specimens, no comprehensive studies of the morphology of post-therapy esophageal cancer exist.
**Design:** 40 esophagectomy specimens removed after chemotherapy and radiation were examined by two pathologists. The gastroesophageal junction, ulcer, scar or gross tumor were entirely submitted for microscopy with margins, lymph nodes and random sections. 17 morphologic features of the primary tumors and 7 features of lymph nodes were scored on a binary (yes/no) or 0-3+ scale. The data comprised 3 categories: Tumor Histology (volume, pleomorphism, neuroendocrine cells, necrosis, presence and volume of mucin pools, lymphovascular and perineural invasion, surgical margins), Stromal Response (degree and depth of fibrosis, calcifications, extent and type of inflammatory response), and Mucosal Changes (ulcer, intestinal metaplasia, dysplasia). Lymph node features included total number, number positive, tumor volume, lymphopenia, fibrosis, necrosis and calcifications. Tumors were staged using AJCC (2002) guidelines. Histologic features were correlated with clinical outcome using Fisher's exact test.
**Results:** The patient group was composed of 6 women and 34 men, with an average follow up of 15 months (range, 1-81). 13 patients died of disease, 3 patients are alive with disease, 19 are alive free of disease and 5 died of other causes. Pathologic stage was the only factor that independently correlated with outcome (p=0.02). As stage increased from 0-4, the likelihood of disease recurrence increased from 11-100%. As degree of fibrosis increased, the likelihood of being alive without disease decreased from 70-40% (p= 0.04). However, fibrosis was not significant when corrected for stage. The presence of acellular mucin pools did not affect outcome. No relationship was found between the other parameters and outcome.
**Conclusions:** This comprehensive study documents the morphology of treated esophageal cancer in a large number of resections. Although numerous treatment related effects were observed, none of them predicted recurrence or death from disease. Post-treatment AJCC stage was the only parameter that was significantly related to outcome. These findings highlight the importance of accurate staging in post therapy resections.

### 516 Mucin Profiling in Signet-Ring Cell Carcinomas

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**Background:** Signet-ring cell carcinomas (SRCCs) are poorly differentiated mucin-producing adenocarcinomas that may arise from many different organs but all share identical histomorphology. Therefore, it is impossible to determine the origin of a metastatic SRCC based on histomorphologic features alone. Mucins are high-molecular-weight glycoproteins that are expressed differentially in different types of glandular epithelia and corresponding adenocarcinomas. Mucin profiling has been applied successfully to identifying the origin of metastatic adenocarcinomas in several studies. In this study, we characterized the mucin profiles of SRCCs from different sites using immunohistochemistry and applied the site-specific patterns to metastatic SRCC to predict primaries.
**Design:** 47 SRCCs, including 38 primary (stomach, 21; colorectum, 11; and breast, 6) and 9 metastases were retrieved from the archival files. Consecutive 4-m m thick sections of a representative formalin-fixed paraffin-embedded tissue block of arch case were immunostained with monoclonal antibodies against MUC1, MUC2, MUC4, MUC5AC (MUC5), and MUC6. Staining was defined as negative (<5%), focal (5-25%), and diffuse (>25%) based on the percentage of positive tumor cells. Diffuse staining was used to construct mucin profiles as MUC+/MUCv/MUC- for consistent, variable, and negative MUC expressions, respectively.
**Results:** Gastric SRCCs were variably positive for MUC1 (9.5%, 2/21), MUC2 (38.1%, 8/21), MUC4 (33.3%, 7/21), MUC5 (5/21, 23.8%), and MUC6 (5/21, 23.8%). Colorectal SRCCs were consistently positive for MUC2 (100%, 11/11) and MUC4 (81.8%, 9/11), variably positive for MUC5 (9.1%, 1/11), and negative for MUC1 and MUC6. Breast SRCCs were consistently positive for MUC1 (100%, 6/6), variably positive for MUC2 (33.3%, 2/6) and MUC6 (16.7%, 1/6), and negative for MUC4 and MUC5. The mucin profiles for gastric, colorectum, and breast were MUC1·2·4·5·6v, MUC2·4+/MUC5v/MUC1·6-, and MUC1+/MUC2·6v/MUC4· 5-, respectively. The mucin expression patterns of the 9 metastatic SRCCs of gastric (2 ovary, 1 omentum, 1 colon), colorectal (1 lymph node, 1 omentum, 1 ovary), and breast (1 skin, 1 lymph node) origin were identical to the mucin profiles of their respective primaries.
**Conclusions:** SRCCs of the stomach, colorectum, and breast have distinct mucin expression profiles, which are maintained in the metastases. Therefore, mucin profiling may be useful to identify the origin of a metastatic SRCC of unknown primary.

### 517 Characteristic and Unusual Pathological Features of a New Familial GIST Kindred

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**Background:** Familial gastrointestinal stromal tumour (GIST) is a rare autosomal dominant genetic disorder. A 38 year old man underwent subtotal gastrectomy, radical pancreaticoduodenectomy, extended right hemicolectomy and partial hepatectomy. Histology of the resection specimen showed features common to previously reported cases of familial GISTs: multifocal tumours with associated hyperplasia of interstitial cells of Cajal (ICCs).

**Design:** Our aim was to document the pathological and molecular features of a new case of familial GIST. Interviews with family members were conducted and a pedigree showing affected family members was constructed. Archived pathology material of family members was reviewed and gene mutation screening was performed using paraffin blocks of from the index case.
**Results:** A point mutation in exon 17 of the *KIT* gene leading to substitution of aspartic acid with tyrosine at position 820 (D820Y) was identified in both normal and tumour tissues from the index patient. One malignant lesion from this patient showed loss of the remaining wild-type *KIT* allele. Review of archived pathology material of family members showed one or more GISTs affecting three generations. The GIST morphologies ranged from epithelioid to spindle cell, and some tumours had prominent skeinoid fibres. The tumours showed a wide range of malignant potential. ICC hyperplasia was present in macroscopically normal small intestine, appendix and colon. One family member had small intestinal diverticular disease complicated by recurrent perforations.
**Conclusions:** This is the second kindred identified with a germline *KIT* D820Y mutation. The range of pathological features in affected patients overlaps with that seen in sporadic GISTs. We postulate that the recurrent perforation of small intestinal diverticular disease seen in one family member may be a consequence of gastrointestinal dysmotility due to ICC hyperplasia. The key clinicopathological features suggesting familial GIST are the involvement of multiple family members, multifocal GISTs and the presence of ICC hyperplasia in macroscopically normal tissue.

### 518 Is Telomere Length Shortening a Predictive Biomarker for Cancer Risk in Ulcerative Colitis?

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**Background:** Neoplastic progression in Ulcerative Colitis (UC) is accompanied by genetic abnormalities and telomere shortening is a candidate for producing such genomic instability. Our overall goal is to examine if telomere shortening can distinguish UC patients at greatest risk of dysplasia/Colorectal Cancer (CRC). We hypothesize that continuous telomere length shortening may correlate with UC progression.
**Design:** Telomere length measurements were assessed using Quantitative Fluorescence In Situ Hybridisation (Q-FISH) and confocal microscopy in longitudinal biopsies from 16 UC patients without dysplasia/CRC and 14 UC patients who progressed to dysplasia and 5 to CRC. Approximately 8 images were analysed for each case.
**Results:** There was no statistical difference in average telomere lengths comparing flat versus polyploid dysplasia cases. Telomere lengths in dysplasia and cancer lesions did not show significant evidence of telomere shortening. 2 of 14 dysplasia patients did however show telomere shortening in previous surveillance biopsies prior to diagnosis of dysplasia. 3 of 16 UC patients without dysplasia or CRC displayed a statistically significant decline in telomere lengths in longitudinal follow up biopsies.
**Conclusions:** Telomere length measurements may be used as a useful screening tool in identifying patients at high risk of CRC development and may require more extensive surveillance.

### 519 Family History of Sporadic Colorectal Cancer Modifies Risks for Specific Molecular Pathology in Tumors: Data from Prospective Cohort of 86,220 Women

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**Background:** A family history of sporadic colorectal cancer (CRC) in a first-degree relative elevates an individual's risk of CRC by ~2 fold. However, the influence of a family history of sporadic CRC on risks for specific molecular alterations in CRC is yet to be fully characterized.
**Design:** We prospectively examined the association between specific genetic alterations in CRC and a history of sporadic CRC in first degree relatives among 86,220 women in the Nurses' Health Study with >25 years of follow-up. Among 254 incident cases analyzed to date, MSI/18q loss of heterozygosity (LOH) and *KRAS/BRAF* mutations were detected by PCR of microsatellites and PCR followed by sequencing, respectively. *TP53* (p53) mutation was assessed by immunohistochemistry.
**Results:** Compared to participants without a family history, the age-adjusted relative risk (RR) for individuals with 1 or more affected first-degree relative(s) was 2.40 (95% CI, 1.39-4.14) for MSI-H cancers and 1.65 (95% CI, 1.13-2.36) for non-MSI-H tumors. Compared to those without a family history, the RR for participants with 2 or more affected first-degree relatives was 8.14 (95% CI, 3.76-17.6) for MSI-H cancers and 2.04 (95% CI, 0.77-5.38) for non-MSI-H cancers. For participants with 1 or more affected first-degree relative(s), the RR was 1.99 (95% CI, 1.42-2.79) for *KRAS* wild-type cancers and 1.38 (95% CI, 0.75-2.54) for *KRAS* mutant cancers. In contrast, the influence of family history did not differ according to the presence or absence of *TP53* or *BRAF* mutation, or 18q LOH.
**Conclusions:** A history of sporadic CRC in a first-degree relative is associated with a higher risk for MSI-H cancers than for non-MSI-H cancers and a higher risk for *KRAS* wild-type cancers than *KRAS*-mutated cancers.

### 520 Activating Mutation of the EGFR Gene Is Rare and Not Responsible for Gefitinib Sensitivity in Colorectal Cancer

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**Background:** Recently activating mutations of the epidermal growth factor receptor (*EGFR*) gene were discovered in non-small cell lung cancers sensitive to gefitinib, EGFR tyrosine kinase inhibitor, but not in gefitinib-resistant cancers. *EGFR* mutations may also play a role in gefitinib sensitivity for advanced colorectal cancers.
**Design:** We analyzed the *EGFR* gene and product as well as the *KRAS* and *BRAF* genes in 22 cases of colorectal cancer. All of the 22 patients have previously untreated metastatic colorectal cancers and were treated with the same combination chemotherapeutic regimen (irinotecan, leucovorin, and 5-fluorouracil) plus daily oral gefitinib. We extracted DNA from paraffin blocks of resected tumors, and sequenced the *EGFR*, *KRAS*, and *BRAF* genes. We also performed immunohistochemistry for EGFR.
**Results:** We found a novel mutation (G724S) in exon 18, the critical kinase domain of *EGFR* in only one chemo-resistant tumor. No *EGFR* mutation was found in any of the other tumors, including 7 other chemo-resistant tumors, 5 chemo-sensitive tumors, and 9 tumors with unknown response data. The *EGFR* mutated tumor showed wild-type *KRAS* and *BRAF*, and weak EGFR expression.
**Conclusions:** Activating *EGFR* mutations are rare and not likely responsible for gefitinib sensitivity in colorectal cancer.

### 521 Expression of Matrix Metalloproteinases (MMP) 2 and 9 in Colorectal Adenocarcinoma

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**Background:** The MMPs 2 (gelatinase A) and 9 (gelatinaseB) belong to the family of endopeptidase enzymes, which regulate the degradation of extracellular matrix components. Relative MMP expression has been linked to cancer invasion, metastases and clinical outcome in a variety of tumor types. The prognostic significance of expression of MMP2 and MMP9 proteins in colorectal adenocarcinoma has not been widely studied.
**Design:** Formalin-fixed, paraffin-embedded sections from 101 colorectal adenocarcionomas were immunostained by antibodies against MMP2 and MMP9. Staining was semiquantitatively evaluated based on both intensity and distribution (weak/focal 1-10%, moderate/intermediate 11-50% and strong /diffuse, 51-100%). The staining pattern was correlated with histologic and prognostic variables.
**Results:** Average tumor cell cytoplasmic immunoreactivity for MMP2 was 79% and MMP9, 78%. Overexpression was more common in males than females, MMP9 (p=0.028) and MMP2 (p=0.071). Advanced Astler Coller tumor stage (C, D) correlated with tumor overexpression of MMP9 (p=0.04) and MMP2 (p=0.006). Increased MMP2 expression was associated with increased lymph node involvement (p=0.03). Increased MMP9 expression correlated with a worse overall survival (p=0.03). Neither MMP2 nor MMP9 expression correlated with vascular invasion or tumor histologic grade.
**Conclusions:** Strong overexpression of MMP9 and MMP2 is associated with advanced tumor stage and strong overexpression of MMP2 is associated with lymph node metastases in colorectal cancer. Dysregulation of MMP activity may play a role in colorectal cancer biology. Further study of these invasion associated proteases in colorectal cancer appears warranted.

### 522 Esophageal and Gastric Carcinoma - Incidence Trends in Ontario, Canada

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**Background:** The increasing incidence of esophageal and proximal gastric adenocarcinomas and the decreasing incidence of distal gastric adenocarcinomas has been documented in several populations. There are no Canadian data to date. The aim of this study was to examine the trends in incidence of these neoplasms in Ontario, Canada's most populous province, over a 39-year period.
**Design:** Analyses were based on data obtained from the Ontario Cancer Registry of Cancer Care Ontario. Numbers of cases and rates per 100,000, age-adjusted to the 1996 Canadian standard were obtained for all esophageal and gastric carcinoma cases reported between 1964 and 2002. Rates were grouped into 5-year periods to analyze trends over the 39-year period. Point and 95% confidence interval estimates of average annual percentage change in incidence rates were calculated with a log-linear regression model.
**Results:** The incidence of adenocarcinoma of the distal esophagus increased in males (average annual increase 3.5%) and decreased in females (average annual decrease 4.8%). The incidence of adenocarcinoma of the cardia increased both in males and females (average annual increase 7.3% (males); 5.1% (females)). The incidence of adenocarcinoma of the cardia and distal esophagus (combined) increased in males (average annual increase 5.4%), with no change in females. The incidence of adenocarcinoma of the antrum and pylorus increased both in males and females (average annual increase 4.2% (males); 5.3% (females)). The incidence of squamous cell carcinoma in the distal and middle thirds of the esophagus remained stable; the incidence in females decreased slightly in the middle third.
**Conclusions:** There has been a significant increase in incidence of adenocarcinomas around the gastroesophageal junction in males over the 39-year study period, as reported in other populations. The increase in incidence of distal gastric adenocarcinomas is unexpected and presently unexplained.

### 523 CDX2 Expression in 259 Gastric Carcinomas-Its Relationship with Mucin Phenotypes and Clinicopathological Characteristics

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**Background:** CDX2 is an intestinal transcription factor that might be involved in the regulation of proliferation and differentiation of intestinal epithelial cells.
**Design:** The purpose of this study was elucidate CDX2 expression and its relationship with mucin phenotypes (as revealed by MUC2, MUC5A, MUC6 and CD10 immunohistochemistry) and clinicopathologic characteristics in 259 gastric carcinomas (including 13 cases of fresh frozen tissue) using Laser Captured Microdissection (LCM) with Real-time RT-PCR and immunohistochemistry.

**Results:** LCM with Real time RT-PCR using fresh frozen tissue showed that intestinal type of gastric carcinomas and intestinal metaplasia had higher CDX2 mRNA than diffuse type of gastric carcinomas and normal foveolar epithelium. Immunohistochemical stain for CDX2 revealed that expression of CDX2 was significantly higher in antral located, old-aged, intestinal-type gastric carcinomas. Also, lower CDX2 expression was related with advanced gastric cancer and lymph node metastasis. Increased intestinal type mucins (MUC-2, CD10) and decreased gastric mucin (MUC5AC) are associated with higher CDX2 expression in gastric carcinomas.
**Conclusions:** These results suggest that CDX2 might play important roles in human gastric carcinogenesis and might be a clinically useful to predict outcome in patients with gastric carcinomas.

### 524 Clinicopathologic Characteristics, Microsatellite Instability, Mucin Core Protein and p53 Protein Expression of Colorectal Mucinous Adenocarcinomas in Relation to Location

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**Background:** Mucinous adenocarcinoma is a well-defined histologic subtype of colorectal cancer and shows some definite clinical features. However, few studies investigate mucinous adenocarcinoma of colorectum in relation to location. Thus, this study was performed to compare the clinicopathologic characteristics, microsatellite instability (MSI), mucin core protein and p53 protein expression of colorectal mucinous adenocarcinoma in relation to tumor location.
**Design:** Sixty nine consecutive colorectal mucinous adenocarcinomas and seventy one non-mucinous adenocarcinomas were included in this study. The clinicopathologic features, including age, sex and stage, were compared in mucinous and non-mucinous adenocarcinoma in relation to tumor location. The MSI status was determined by fluorescent-labeled sequencing analysis of BAT26 and immunohistochemical staining of hMLH1 and hMSH2. The expression of MUC1, MUC2, MUC5AC and p53 were assessed by immunohistochemistry.
**Results:** Mucinous adenocarcinomas were characterized by large tumor size (P=0.002), right-sided location (P=0.019), association with serrated adenoma (P<0.001), MSI (P=0.012), MUC2 expression (P<0.001), MUC5AC expression (P<0.001), low MUC1 (P=0.004) and low p53 expression (P<0.001), compared with non-mucinous adenocarcinomas. In relation to tumor location and histology, right-sided non-mucinous adenocarcinomas were associated with old age (P=0.004), MUC2 (P=0.025) and MUC5AC expression (P=0.005) and low p53 protein expression (P=0.046), compared with left-sided non-mucinous adenocarcinomas. Right-sided mucinous adenocarcinomas, when compared with left-sided mucinous adeocarcinomas, were characterized by peritumoral lymphocytic response (P=0.008), MSI (P=0.003), MUC2 expression (P=0.012), MUC5AC expression (P=0.010), low p53 protein expression (P= 0.013) and association with serrated adenoma (P=0.009).
**Conclusions:** This study showed that MUC2 and MUC5AC expression and low p53 expression were associated with right-sided tumor location as well as mucinous histology. And MSI, peritumoral lymphocytic response and association with serrated adenoma were associated with right-sided mucinous adenocarcinoma. Our results indicate that mucinous adenocarcinoma is a distinctive entity of colorectal cancer but shows different phenotypes in relation to tumor location.

### 525 Mutational Analysis of Neosquamous Epithelium in Barrett's Esophagus Suggests Heterogenous Cells of Origin in Most Cases

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**Background:** Patches of apparently normal "neosquamous epithelium" (NSE) can arise in Barrett's esophagus (BE) segments during acid suppression by proton pump inhibitors or as a result of ablation therapy. Morphologic studies have suggested that NSE may develop from either encroachment of adjacent squamous epithelium at the squamocolumnar junction, extension of epithelium from the submucosal gland ducts or from a pluripotent stem cell with the potential for either squamous or columnar differentiation. NSE arising from a pluripotent stem cell is of clinical concern because the stem cell might contain mutations commonly detected in "BE", such as in p16 or p53, and this may be indicative of malignant potential. The aim of this study was perform mutational analysis of NSE in BE containing mutations to determine whether or not a common progenitor exists for these two types of epithelium.
**Design:** Patches of NSE and surrounding intestinalized epithelium from 21 patients with BE (M/F ratio: 6.1, mean age: 73.7 yrs, mean length of BE: 7.9 cm) were evaluated for mutations in exon 2 of p16/CDKN2a or exons 5-9 of the p53 gene by sequencing PCR amplified genomic DNA. NSE and Barrett's epithelium were isolated by microdissection. NSE were verified to be free of Barrett's epithelium and its DNA was obtained for sequence analysis of p16 and p53 (Big Dye Terminator v3.0, ABI).
**Results:** 9/21 BE patients showed mutations in p16 (point mutations or deletions), and 12/21 showed mutations in p53 (point mutation) within Barrett's epithelium. 2/20 foci of NSE had mutations in either p16 or p53 identical to those found in the surrounding Barrett's epithelium. However, one of these mutations, in p53, was subsequently found in DNA from blood and represents a constitutive mutation. The remaining mutation in the NSE, a 146 bp deletion in p16, was verified by a second independent analysis and, thus, is unlikely to have arisen independently in both Barrett's epithelium and NSE.
**Conclusions:** Our mutational data supports the hypothesis that, in most circumstances, separate stem cell compartments exit for the generation of NSE and Barrett's epithelium in patients with BE. However, this study also provides evidence in support of the existance of a pluripotent stem cell population that is capable of differentiating into both intestinal epithelium or NSE.

### 526 EGFR Immunohistochemical Expression in Microsatellite Stable and Unstable Colorectal Carcinomas

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**Background:** Colorectal carcinomas (CRC) with microsatellite instability (MSI) have been described as having distinctive clinicopathologic characteristics. It has been suggested that, patients with MSI colorectal tumors do not benefit from 5-FU-based chemotherapy. With the introduction of novel targeted therapies directed against Epidermal Growth Factor Receptor (EGFR), there is an increasing interest in immunohistochemistry (IHC)-based EGFR to select cancer patients eligible for specific treatment. EGFR is expressed in 23-75% of CRC tumors, but differences between MSI and stable CRC have not been previously studied.
**Design:** Formalin-fixed, paraffin-embedded tissues from 19 MSI tumors and 107 MSS tumors were stained with EGFR (EGFR pharmDx™ Kit, Dakocytomation). Staining was scored for intensity (0, 1+, 2+ or 3+) and percentage of positive tumor cells. Positive cases were considered when membrane staining was observed in ≥1% of tumor cells at any intensity. The results were correlated with clinicopathological features (age, sexr, grade, histological subtype, TNM stage, and location)
**Results:** Positive staining for EGFR was observed in 12/19 (63.2%) of MSI tumors and in 29/107 (26,9%) of stable tumors (p=0,002). Strong expression (2 or 3+, >10%) was observed in 7/19 (36.8%) of MSI tumors and in 10/107 (9.3%) of stable tumors (p=0,001). We observed a trend toward higher levels of EGFR expression in cases with MLH1 loss of expression compared with those with MSH2 loss of expression (73.3% vs. 25%, respectively; p=0,086). There was not statistical association with the clinicopathological features evaluated.
**Conclusions:** MSI tumors are more frequently positive for EGFR than stable tumors. Therefore, the benefit of anti-EGFR therapies will be probably higher in patients with MSI CRC tumors.

### 527 CDX-2 Expression in Upper Gastrointestinal Tract Adenocarcinomas

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**Background:** CDX-2 is a homeobox gene that expresses an intestinal-specific transcription factor which is highly sensitive and specific for colonic and small intestinal adenocarcinomas. Recent studies have shown that CDX-2 can also be expressed in intestinal metaplasia (IM) of the stomach and gastric adenocarcinomas associated with IM, but not in the normal gastric mucosa. The aim of this study was to investigate the diagnostic value of CDX-2 expression in adenocarcinomas of esophagus, stomach and gastro-esophageal (GE) junction and the possible value in determining an upper GI tract primary in the work-up of metastatic malignancies of unknown primary.
**Design:** Sixty surgically resected adenocarcinomas of esophageal, GE junction and gastric origin were analyzed. Immunohistochemical staining for CDX-2, CK7, and CK20 was performed with appropriate positive and negative controls. Intranuclear staining constituted a positive result for CDX-2.
**Results:** IM was present in the background benign mucosa of 30 cases, including 16/30 GE junction, 10/15 esophageal and 4/15 gastric carcinomas. The overall CDX-2 expression was seen in 57% (34/60) of the adenocarcinomas, including 17/30 GE junction, 11/15 esophageal, and 6/15 gastric origin. 19/30 (63%) of adenocarcinomas with IM and 11/30 (48.25%) of adenocarcinomas without IM showed positive CDX-2 expression. 10/16 (63%) of adenocarcinomas arising from Barrett's esophagus were positive for CDX-2. The majority of the adenocarcinomas from all three sites expressed CK7 (77%), including 22/30 GE junction, 12/15 esophageal and 12/15 gastric carcinomas. 5 GE junction and 3 gastric carcinomas were CK7-/CK20+
**Conclusions:** We demonstrated that gastric, esophageal and GE junction adenocarcinomas frequently express CDX-2, but less than the reported incidence in colorectal and small intestinal adenocarcinomas. In our experience, CDX-2 expression is seen in a slightly higher percentage of esophageal and GE junction adenocarcinomas associated with intestinal metaplasia than without IM. In a metastatic adenocarcinoma, co-expression of CDX-2 and CK7 strongly suggest upper GI tract as the primary site.

### 528 Concordant Loss of MTAP and p16/CDKN2A Expression in Barrett Esophagus and Adenocarcinoma: Evidence of Homozygous Deletion in Non-Invasive Precursor Lesions

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**Background:** The gene that encodes methylthioadenosine phosphorylase (MTAP), an enzyme involved in adenine and methionine salvage pathways, is located in the 9p21 chromosomal region telomeric to the p16INK4A/CDKN2A tumor suppressor gene (p16). Inactivation of p16 occurs by three different mechanisms: hypermethylation of the gene promoter, intragenic mutation coupled with loss of the second allele, and homozygous deletion. Immunohistochemical labeling for the p16 gene product parallels gene status but does not elucidate the mechanism of gene inactivation. Since MTAP is often co-deleted with p16, concurrent immunolabeling for both proteins can identify cases with homozygous p16 deletion.
**Design:** Tissue microarrays (TMAs) were constructed from 79 cases of Barrett esophagus and/or Barrett-associated adenocarcinoma comprising 325 individual cores. Multiple cores were arrayed from any given case, and when available, included the entire histologic spectrum of Barrett-associated intestinal metaplasia-dysplasia-carcinoma. TMAs were labeled with monoclonal antibody against MTAP protein (clone 6.9, Salmedix, Inc) and p16 (Clone 16P07, Neomarkers). Complete loss of labeling was considered negative, while any labeling (p16: nuclear; MTAP: cytoplasmic) was considered positive.

**Results:** The absence of MTAP labeling occurred exclusively in conjunction with the absence of p16 labeling, confirming that the p16 gene is indeed the target of 9p21 deletions. Lack of MTAP and p16 expression was seen in 26 cores from 22/79 (28%) cases, including 15 cores with adenocarcinomas and 11 with precursor lesions, comprising 4 non-dysplastic Barrett esophagus, 4 low grade dysplasias, and 3 high grade dysplasias. Of the 15 MTAP-, p16- cancers, concurrent loss of expression in the precursor lesion, including in non-dysplastic Barrett esophagus, was seen in 5 cases.
**Conclusions:** Concurrent MTAP and p16 negativity can serve as a convenient surrogate for p16 gene homozygous deletion in archival tissues. Inactivation of p16 by homozygous deletion appears to be an early event in Barrett adenocarcinoma, occurring in non-invasive precursor lesions, including non-dysplastic Barrett mucosa, in subsets of cases. In the absence of MTAP, cells depend exclusively on the *de novo* synthesis pathway for production of adenosine. This loss of MTAP during 9p21 homozygous deletion may be exploited therapeutically using a nucleoside analog.

### 529 Loss of HepPar-1 Antibody Reactivity as a Marker of Dyspastic Barrett's Mucosa and Esophageal Adenocarcinoma

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**Background:** Hepatocyte Paraffin-1 (HepPar-1) antibody was recently shown to react with intestinal metaplastic epithelium seen in Barrett's esophagus, whereas HepPar-1 staining is lost in esophageal adenocarcinoma. Staining properties and diagnostic utility of HepPar-1 in dysplastic Barrett's mucosa have not been addressed. The goal of the present study was to expand on the original observations using a larger sample size and to evaluate staining properties of HepPar-1 in cases morphologically diagnostic or equivocal for dysplasia.
**Design:** A total of 224 cases were analyzed by immunohistochemical staining with HepPar-1 (DAKO) antibody utilizing primarily tissue microarrays constructed from esophago-gastrectomy specimens. We analyzed 16 cases of Barrett's esophagus, 46 cases of dysplastic Barrett's mucosa (27 high grade, 13 low grade, and 6 indefinite for dysplasia), 68 cases of esophageal adenocarcinoma (38 moderately to poorly, 17 moderately, and 7 well to moderately differentiated), 52 cases of normal gastric mucosa and 42 cases of normal esophageal mucosa.
**Results:** All examined cases of non-dysplastic Barrett's mucosa were strongly HepPar-1 positive, whereas staining of normal gastric and esophageal mucosa was negative. Overall, 95% of esophageal adenocarcinomas were either negative or stained weakly with HepPar-1. All cases of moderately to poorly differentiated carcinomas were either negative (95%) or stained weakly (5%). With a single exception, moderately differentiated carcinomas were either negative (65%) or stained weakly (29%). Well to moderately differentiated carcinomas were either negative or stained weakly with HepPar-1 in 14% and 57% of cases, respectively. Overall, 94% of dysplastic Barrett's mucosa stained either negatively or weakly with HepPar-1. High grade dysplasias were negative or weak in 78% and 15% of cases, respectively, and low grade dysplasias were negative or weak in 62% and 31% of cases, respectively. In our material, all cases morphologically diagnosed as indefinite for dysplasia were either negative (67%) or weak (33%) for HepPar-1, suggesting that these cases represent a truly dysplastic process.
**Conclusions:** HepPar-1 is a sensitive and specific marker of Barrett's mucosa, whereas HepPar-1 staining is negative or weak in the vast majority of esophageal adenocarcinomas as well as dysplastic Barrett's mucosa. HepPar-1 is a useful diagnostic tool in cases morphologically equivocal for dysplasia.

### 530 Claudin-1 Is a Strong Prognostic Indicator in Stage II Colonic Cancer: A Tissue Microarray Study

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**Background:** Tight junction (TJ) associated proteins are key molecular components governing cellular adhesion, polarity and glandular differentiation. TJ proteins also play critical roles in cellular proliferation and neoplastic pathways via their functions as couplers of the extracellular milieu to intracellular signaling pathways and the cytoskeleton. Neoplastic cells frequently exhibit structural and functional deficiencies in the TJ. The purpose of this study was to determine the pattern of expression and prognostic value of four TJ associated proteins, claudin-1, claudin-4, occludin and ZO-1 in a cohort of TNM stage II colon cancer using tissue microarray technology.
**Design:** In this study, we retrospectively analyzed, resected and otherwise untreated paraffin embedded specimens from 129 consecutive patients with TNM stage II colonic carcinomas for claudin-1, claudin-4, occludin and ZO-1 protein expression by immunohistochemistry.
**Results:** Seventy five, 58, 56 and 44% of the tumors exhibited high expression levels (+2 and +3 immunopositivity) of claudin-1, claudin-4, occludin and ZO-1 respectively. Low expression levels of claudin-1 and ZO-1 were directly associated with higher tumor grade (P=0.05 and 0.03 respectively). Multivariate analysis indicated that lymphovascular invasion (P= 0.01) and low levels of claudin-1 (P=0.0001) expression were independent predictors of recurrence and that reduced claudin-1 expression (P= 0.0001) was associated with poor survival. A strong direct correlation was seen between levels of claudin-1 and claudin-4 expression (P=0.0001) and between levels of occludin and ZO-1 expression (P=0.001).
**Conclusions:** This study is the first to comprehensively examine the expression of several TJ associated proteins in colonic neoplasms and to correlate their expression with disease progression. Loss of claudin-1 expression proved to be a strong predictor of disease recurrence and poor patient survival in stage II colon cancer.

### 531 MSH-2, MCM7 and KI-67 Are Useful Markers in the Diagnosis of Barrett's Associated Neoplasia

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**Background:** Barrett's esophagus (BE) and Barrett's related dysplasia (BRD) are markers of increased risk for esophageal adenocarcinoma. BRD may be focal, and difficult to diagnose on H&E. We studied human mismatch repair protein 2 (MSH2), minichromosome maintenance protein 7 (MCM7) and the proliferation marker KI-67 in an attempt to establish a helpful immunohistochemical panel in diagnosing Barrett's-related neoplasia.
**Design:** Forty-two esophageal biopsies were retrieved from the archives, including 14 cases of BE without dysplasia or carcinoma, 11 Barrett's-related LGD (BRLGD), 7 Barrett's-related HGD (BRHGD), and 10 Barrett's-related adenocarcinoma (BRAC). H&E slides were reviewed, tissue blocks were re-cut, and immunohistochemistry performed for MSH2 (mouse monoclonal, 3.3 m g/ml, NovoCastra, Newcastle upon Tyne, UK), MCM7 (mouse IgG1, 0.04m g/ml, Dako Corp., Carpinteria, CA) and KI-67 (MU297-UC, 900 m g/ml, BioGenex, San Ramon, CA) using an Envision+ detection system (Dako) with isotype matched IgG1 negative controls. Staining distribution was determined by assessing percentage of cells staining positively, defined as: >75% = diffuse, >25% and <75% = intermediate, >5 and <25% = focal, and <5 = minimal to no staining. Cases were scored 0-3 based on staining intensity.
**Results:** The BE-only group showed no to minimal reactivity to MCM7; MSH2 and KI-67 showed minimal to intermediate positivity, 57% and 6% of cases with full thickness staining, respectively. BRLGD showed focal to intermediate reactivity for MCM7 and KI-67, 20% and 53% of cases with full thickness staining, respectively; MSH2 showed intermediate to diffuse positivity, 80% of cases with full thickness reactivity. BRHGD showed intermediate reactivity for MCM7 and KI-67, each with 83% of cases showing full thickness staining; MSH2 showed intermediate to diffusely strong staining, with full thickness reactivity in 100% of cases. BRAC stained similarly to the BRHGD group for all three markers.
**Conclusions:** MSH2, MCM7 and KI-67 is a useful panel in BRD and BRAC. Increased full thickness expression of MSH2 and MCM7, and increased surface expression of KI-67, are seen in BRLGD as compared to BE-only. Compared to BRLGD, MCM7 and KI-67 show increased full thickness expression and surface expression in BRHGD, respectively. Thus, this is a useful panel in confirming the diagnoses of BRD and BRAC, and could be helpful in distinguishing BRLGD from BRHGD.

### 532 Expression of Tumor Suppressor 14-3-3 σ Protein in Anal Squamous Intraepithelial Neoplasia

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**Background:** The 14-3-3 σ is a p53-regulated G2/M inhibitor involved in numerous cellular signaling pathways related to cell cycle, DNA repair and apoptosis. In addition to its G2/M arrest function, 14-3-3 σ also inhibits apoptosis through interactions with pro-apoptotic proteins such as Bax and BAD. Thus, it appears that while 14-3-3 σ halts cell cycle progression at the G2 checkpoint, it also inhibits apoptosis, possibly to allow for cell repair. Recent studies showed that 14-3-3 σ was silenced transcriptionally through promoter hypermethylation mainly in HPV-negative vulvar squamous cell carcinoma (SCC). However, the expression of 14-3-3 σ protein has not yet been studied in anal/perianal SCC and its precursors.
**Design:** We evaluated the expression of 14-3-3 σ, p16 and p53 in 5 cases of normal perianal squamous mucosa, 5 cases of squamous hyperplasia and 20 cases of AIN, including 16 of bowenoid type and 4 of differentiated type. Bowenoid AIN includes both basaloid and warty types, both of which are frequently seen in HPV-related squamous dysplasia. Differentiated type AIN lacks HPV-related changes and morphologically is similar to the differentiated type of vulvar intraepithelial neoplasia.
**Results:** Normal squamous epithelium showed no immunoreactivity for 14-3-3 σ. Squamous hyperplasia showed weak (1+) submembrane cytoplasmic staining. Overexpression of 14-3-3 σ was found in 95% (19/20) of AIN cases. 14-3-3 σ staining was strong (4+), moderate (2+-3+) and weak (1+) in 10 (50%), 8 (40%) and 1 case (5%), respectively. The pattern of staining was distinctive and was typically perinuclear cytoplasmic and/or nuclear. There was no significant difference in staining pattern and intensity between bowenoid and differentiated AIN. Expression of 14-3-3 σ was independent of p53 and p16 expression. Interestingly, p53 was diffusely expressed in the basal and suprabasal zone in all 4 cases of differentiated AIN, but was patchy and focally expressed in 8 of 16 cases (50%) of bowenoid AIN. In contrast, p16 was expressed in 15 of 16 (94%) bowenoid AIN, but in none of the cases of differentiated AIN.
**Conclusions:** Our findings indicate that 14-3-3 σ is overexpressed in 95% of AIN lesions. The 14-3-3 σ protein relocalizes from a submembranous cytoplasmic location in squamous hyperplasia to a perinuclear or intranuclear location in squamous dysplasia. Overexpression of 14-3-3 σ is seen in both bowenoid and differentiated AIN and is independent of the expression of p53 and p16. Detection of 14-3-3 σ, in conjunction with p16 and p53, may be useful in the early detection of AIN.

### 533 Immunohistochemical Expression of Fatty Acyd Synthase (FAS) in GIST

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**Background:** Fatty acid synthase (FAS), a key enzyme in de novo lipogenesis, protects cancer cells from apoptosis and is overexpressed in many epithelial tumors that display aggressive biologic behavior. Recently, FAS has been proposed as a prognostic indicator in mesenchymal malignances. Here, we determined FAS expression in gastrointestinal stromal tumors (GISTs).

**Design:** Tissue microarrays (TMAs) were prepared from 60 GISTs. All cases were stratified by the NIH Consensus Conference risk category, and immunostained with CD117 (polyclonal, DakoCytomation), Ki67 (clone MIB-1, DakoCytomation) and FAS (polyclonal,Transduction Laboratories). Four true smooth muscle tumors were included in the study, as controls. Cases were deemed FAS positive when a cytoplasmic staining was seen. Cases were subdivided in two groups, one containing very low, low and intermediate risk GISTs (28 cases) and one containing high risk GISTs (32 cases).
**Results:** FAS was expressed in 36 of 60 GISTs (60%): 12 of 28 low/intermediate risk cases (42%) and 24 of 32 high risk GISTs (75%) ($p<0.03$). FAS immunoreactivity was present in the majority of neoplastic cells. FAS was not expressed in gastric leiomiomas or leiomiosarcomas. Five of 60 cases, four from high and one from low risk GISTs, were positive for Ki67. All of these were FAS positive.
**Conclusions:** FAS is overexpressed in the majority of GISTs (60%), more often in highly proliferative and biologically aggressive types. FAS overexpression may aid in the identification of intermediate/low risk GISTs that will behave aggressively and represents a therapeutic target in this disease.

### 534 Maintenance Infliximab Treatment and Resolution of Histopathologic Abnormalities in Colonic Crohn's Disease

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**Background:** Mucosal histopathologic alterations including crypt architectural distortion, lymphoplasmacytic infiltration and epitheloid granuloma formation are key components in the diagnosis and grading of activity in Crohn's disease (CD). These alterations are felt to be durable and longstanding features of CD. The anti-TNF-a chimeric monoclonal antibody infliximab (Remicade) has improved CD treatment outcomes, with complete mucosal healing in up to 30% of patients. The impact of maintenance infliximab treatment on CD histopathologic features has not been defined. We hypothesized that maintenance infliximab treatment of CD would correlate with significant histologic improvement, and normalization of mucosal architecture.
**Design:** Endoscopic mucosal pinch biopsies in 46 consecutive colonic CD patients before and after maintenance infliximab treatment (average monthly treatments: 11) were retrospectively reviewed. Four quadrant biopsies were performed in anatomic segments of the colon. Pooled pre and post infliximab treatment biopsies were evaluated using a set of standard criteria quantifying ulceration, erosions; crypt destruction, alteration or abcesses; and inflammation using a 1–5 scale. Presence/absence of granulomas was also recorded. The scoring pathologist was blinded to infliximab treatment status. A histologic disease activity index (HDAI; 1–5 score, with higher scores reflecting increased severity) of the pre and post treatment groups was determined by taking the total number of activity events divided by the number of biopsies.
**Results:** 143 pre and post maintenance infliximab treatment biopsies were analyzed. Post-infliximab treated biopsies showed significant improvement in mucosal morphology, and a complete rebuilding of colonic architecture approaching normal was frequently encountered. HDAI decreased from 2.1 to 0.4 following infliximab maintenance treatment ($p=6.4 \times 10^{-5}$). Furthermore, an HDAI of $\leq 2$ was found in 83% of infliximab treated biopsies. HDAI scores of $\geq 3$ were found in 33% of pre and only 17% of post infliximab treated biopsies. Granulomas were detected in 14 pretreatment biopsies, but were found in none of the post-infliximab treated specimens.
**Conclusions:** Maintenance infliximab treatment significantly improved histopathologic features of colonic CD. A subgroup of patients had normalization of crypt architecture, with resolution of granuloma formation, suggesting that infliximab maintenance treatment may reverse histopathologic abnormalities in CD.

### 535 Expression of Claudin 7 and Sox-4 in Gastric and Colorectal Adenocarcinomas

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**Background:** Claudin7, a tight junction protein with structural and regulatory functions, and Sry-type HMG box (sox)4, a transcription factor involved in normal organogenesis, have been identified by microarray analysis as showing temporal variation in expression in embryonic mouse colon. We hypothesized that gastrointestinal malignancies would show altered expression of these proteins.
**Design:** Two tissue microarrays (TMA) were constructed, one containing 106 colon carcinoma (CRC) samples (including 6 medullary, 29 mucinous, and 5 signet ring cell carcinomas) and 12 normal colon cores, the other containing 47 gastric adenocarcinomas (17 diffuse, 30 intestinal) and 82 normal, inflamed, atrophic, or metaplastic gastric mucosa cores. The TMAs were stained for claudin7 (Zymed, 1:400 dilution) and sox4 (Chemicon, 1:200 dilution). Claudin7 membranous staining and sox4 nuclear and cytoplasmic staining were scored as 0 (no/trace), 1+, 2+, or 3+, based on staining intensity and % cells positive.
**Results:** Normal colon showed lateral membrane staining for claudin7; non-neoplastic stomach was largely negative, except for strong expression by endocrine cells and intestinal metaplasia. Sox4 showed 0 to 1+ staining in normal colonic mucosa and 2+ nuclear staining in non-neoplastic stomach. In CRC, there was a significant association with both grade and tumor subtype by claudin7 staining ($p=0.0002$ and $p < 0.0001$, respectively), with reduced staining (0 or 1+) in grade 2 (41/55) and grade 3 (21/28) CRC and in special subtypes. In CRC, 9/12 grade 1 tumors showed low levels of cytoplasmic sox4 expression; grade 2 and 3 cancers were more likely to show 2+ (19/88) or 3+ (27/88) staining ($p=0.0051$). There was no association between sox4 nuclear staining and grade ($p=0.2751$) for CRC. For gastric cancers, 10/12 diffuse and 22/30 intestinal cancers showed aberrantly high (2 or 3+) claudin7 expression. 3+ cytoplasmic expression of sox4 was seen only in intestinal gastric cancers (7/30). There was no association of claudin7 or cytoplasmic or nuclear sox4 expression with grade in gastric cancers.
**Conclusions:** Aberrant expression of claudin7 and sox4 is seen in both gastric and colorectal adenocarcinomas. Reduced expression of claudin7 is more common in higher grade CRC and in special subtypes associated with high microsatellite instability. No association of claudin7 or sox4 expression with tumor grade was noted in gastric adenocarcinomas, although strong cytoplasmic expression of sox4 was seen only in intestinal gastric cancers.

### 536 RASSF1A Expression and Gene Methylation in Ileal Carcinoid Tumors

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**Background:** Ras-association domain family 1A(RASSF1A) is a tumor suppressor gene which is inactivated by methylation in various tumors. The role of RASSF1A in ileal carcinoid tumor pathogenesis is unknown.
**Design:** We examined 44 primary and 52 carcinoid tumors metastatic to the liver and lymph nodes along with normal enterochromaffin (EC) cells in the ileum by immunohistochemistry using tissue microarray (TMA) to determine the role of RASSF1A in carcinoid tumor pathogenesis. A subset of these tumors (n=15) was analyzed by Western blotting and by methylation specific PCR (MS-PCR).
**Results:** RASSF1A was expressed in the normal EC cells of the ileum. Expression of RASSF1A was decreased in metastatic carcinoid tumors compared to the primary tumors in the TMA ($p=0.052$). MS-PCR showed an increase in methylation in metastatic tumors (77%) compared to primary tumors (67%). Western blotting showed decreased protein expression in the metastatic tumors (25%) compared to primary tumors (69%).
**Conclusions:** Epigenetic inactivation of RASSF1A resulting in a decrease in protein expression is more common in metastatic compared to primary carcinoid tumors implicating RASSF1A in the pathogenesis and progression of ileal carcinoid tumors. RASSF1A methylation may be a useful marker in patients with ileal carcinoid tumors.

### 537 The Prognostic Significance of Intratumoral Neutrophils in Advanced Stage Colorectal Adenocarcinoma

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**Background:** Today, several lines of evidence indicate that neutrophils act nonspecifically against tumor cells. However, the correlation between tumor-infiltrating neutrophils (TIN) and clinicopathologic features has not been yet fully elucidated. This study investigates the prognostic influence of TIN in cases of advanced stage colorectal adenocarcinoma.
**Design:** The study included 230 advanced stage colorectal adenocarcinomas resected from equal number of patients: 150M/80F, aged from 36-83 (median 61) years. Tumors were staged according to Astler-Coller system. Patients were followed-up for a period of 40-156 (median 85) months. Paraffin sections (4μm thick) from the tumors were stained with H&E. On them, the number of TIN was assessed in a semiquantitative way using the mean value±SD, of 20 nonoverlapping high-power fields (magnification X400). Thereafter the patients were divided into 2 groups: those with ≥10TIN/20HPF and those with <10 TIN/20HPF. For statistical purposes the univariate and multivariate analysis were employed to assess the effect of the prognostic factors on survival, and the Kaplan-Meier method was used to estimate the survival rates in the two groups.
**Results:** Eighty-five tumors were of stage $B_2$, 121 of stage C and 24 of stage D. Sixty-four patients (41F/23M) had TIN≥10. Univariate analysis revealed that the factors related with higher, disease-free, survival, were: female gender ($p<0.01$), tumor greatest diameter<5cm ($p<0.05$), earlier stage (stage $B_2$ vs. stage C, $p<0.05$), TIN (≥10/20HPF-$p<0.01$), and surgical margins free of neoplastic disease. Further statistical analysis of the possible relation of the clinicopathologic factors with TIN, revealed that female patients with ≥10 TIN/20HPF, displayed 35% lower mortality compared to those with <10TIN/20HPF ($p<0.01$); this could not be demonstrated in male patients ($p>0.05$).
**Conclusions:** The results of this study imply that females appear to have a better prognosis than males in advanced stage colorectal adenocarcinoma. Gender differences in some host defense mechanisms and particularly in neutrophil function, may be responsible for this event. Establishment of these findings, in further studies, would give new insights about host reaction to colorectal cancer growth and, ultimately, may have implications regarding the identification of low-risk patients who could be spared adjuvant therapy.

### 538 MUC1, 2, 5AC, and 6 Expression Pattern in Barrett's Metaplasia, Barrett's Related Dysplasia and Adenocarcinoma

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**Background:** Mucins secreted in the esophagus play an important role in cytoprotection against reflux of gastric contents. Changes in the histochemical characteristic of surface epithelial mucins is the hallmark of Barrett's metaplasia (BM). The aim of the study is to evaluate and compare the immunostaining pattern of MUC1, MUC2, MUC5AC, and MUC6 in normal esophageal mucosa, Barrett's metaplasia, dysplasia and adenocarcinoma.
**Design:** A retrospective collection of paraffin-embedded tissue from seven resection specimens containing normal junctional mucosa, Barrett's metaplasia, Barrett's dysplasia and adenocarcinoma in the same tissue section were identified from pathology files in Creighton University Medical Center. Adenocarcinoma were deemed to be arising from the Barrett's esophagus, if, on histological examination, there was intestinal metaplasia proximal to the tumor. These sections were immunostained with monoclonal antibodies against MUC1, MUC2, MUC5AC, and MUC6.

**Results:** The results are shown in Table 1. All cases showed week superficial staining for MUC1 in normal squamous epithelium. None other mucins were expressed in normal squamous epithelium. All four types of mucins were expresssed in Barrett's metaplasia.
**Conclusions:** MUC1 which is a gastric-type mucin remains expressed throughout phases of progression from BM to adenocarcinoma. This observation in MUC1 staining pattern is in contradiction to other studies that showed low level of expression of this mucin in Barrett's metaplasia and high expression in dysplasia and adenocarcinoma. Intestinal metaplasia of Barrett's esophagus showed high expression of MUC2 which is characteristic of normal intestinal epithelium and it remains expressed through all phases of progression from metaplasia to adenocarcinoma. However, the expression of MUC2 was decreased by 29% in adenocarcinoma. MUC5AC and MUC6, which are gastric-type of mucins, showed good expression in BM and a reduced expression ( ~67% for MUC5AC and ~ 58% for MUC6) with progression to high grade dysplasia and adenocarcinoma.

Table 1

| Stain | Normal squamous mucosa | BM | Dysplasia | Adenocarcinoma |
|---|---|---|---|---|
| MUC1 | 100% | 100% | 100% | 100% |
| MUC2 | 0% | 100% | 100% | 71% |
| MUC5AC | 0% | 100% | 84% | 33% |
| MUC6 | 0% | 100% | 71% | 42% |

### 539 MBD2 in Colon Cancer and Its Association with DNA Methylation

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**Background:** Colonic cancer results from progressive accumulation of genetic epigenetic alterations that lead to transformation of normal colonic epithelium to colon adenocarcinomas. DNA methylation as 5-methylcytidines (5-MeC) and MBD2, a methyl-CpG-binding domain 2 protein, have attracted much attention because of their roles in epigenetic regulation of gene expression. Global hypomethylation in DNA is found in most tumors including colon cancers in animals and human. However, we do not know how early and at what stage(s) in colonic carcinogenesis the DNA hypomethylation occurs. MBD2 has recently been shown to catalyze demethylation by directly removing methyl groups from 5-methylcytosine residues in genomic DNA. In order to gain a better insight into the involvement of MBD2 and DNA methylation in colonic cancer, we investigated the expression of MBD2 and global methylation level of DNA using polyclonal antibodies in hyperplastic, adenomatous polyps and adenocarcinomas of colon.
**Design:** Ten cases of colon adenocarcinoma, adenomatous polyp, and hyperplastic polyp each were retrieved from the Department of Pathology at OSU. Aberrant crypt foci (ACF) were identified associated with either adenomatous polyp and/or adenocarcinoma. Immunohistochemical stains were used to examine the expression of MBD2 and 5-MeC. Antibodies for MBD2 and 5-MeC were from Santa Cruz Biotechnology and Megabase Research Products, respectively. The staining intensity was scored as 1+ to 4+.
**Results:** Both MBD2 and 5-MeC are nuclei staining. For MBD2 staining, all (100%) ACF and 7 out of 10 (70%) adenomatous polyps and adenocarcinomas show 3-4+, while all (100%) hyperplastic polyps and normal mucosa show 1-2+ except the proliferative zone at the base of the crypts showing 3+ positivity. For 5-MeC staining, superficial mature epithelium in normal mucosa were uniformly and intensively stained (3-4+), 8 out of 10 carcinoma (80%) and all (100%) ACF show 1-2+, 6 out of 10 (60%) adenomatous polyps show 2-3+, and 7 out of 10 (70%) hyperplastic polyps show3+ positivity.
**Conclusions:** Our primary results indicate MBD2 was highly expressed in ACF, a very early stage of colonic carcinogenesis, and remain high throughout all the late stages of carcinogenesis. Progressive hypomethylation of DNA was shown in the hyperplastic, adenomatous polyps and adenocarcinomas, which is related to the colonic carcinogenic progression. The results suggested the demethylating feature of MBD2 is associated with global hypomethylation of DNA in the development of colonic cancer.

### 540 Epidermal Growth Factor Receptor Expression and Gene Amplification in Colorectal Carcinoma: An Immunohistochemical and Chromogenic In Situ Hybridization Study

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**Background:** Epidermal growth factor receptor (EGFR) has emerged as a rational target for therapy in colorectal carcinomas (CRCs), and the newly FDA-approved drug cetuximab targets the EGFR specifically. Several trials have shown that cetuximab might improve survival in patients with advanced CRC, however, no correlation has been shown between its efficacy and the EGFR level in the tumor as detected by immunohistochemistry (IHC), the current method of choice for patient selection. Chromogenic in situ hybridization (CISH) is a technique that can be used to evaluate gene amplification on tissue sections. Its use in evaluating EGFR in CRCs has not been explored.
**Design:** Using tissue microarray techniques, comparative analyses of EGFR expression by IHC (DakoCytomation) and gene amplification by CISH (Zymed Inc) were performed on 158 CRCs from 158 patients. IHC results were scored as 0-3+ based on the intensity of membrane staining. The CISH signals were counted in 30 nuclei per tissue core and interpreted as: no amplification, <5 EGFR gene copies / nucleus; low-level amplificaiton, 5-10 copies; and high-level amplification, >10 copies. The Jonckheere-Terpstra test was used to detect the ordered difference among categories.
**Results:** The rate of tissue loss was 7%, yielding 147 analyzable cases: 123 primary, 24 metastatic (6 in liver, 18 in lung). As shown in the Table, 85% (105/123) primary and 79% (19/24) metastatic CRCs showed positive IHC, whereas only 12% (15/123) primary and 8% (2/24) metastatic CRCs showed gene amplification. Although a positive correlation was detected between the intensity of IHC positivity and the likelihood of gene amplification in both the primary (p=0.01) and the metastatic (p=0.05) CRCs, IHC had a low specificity (17% in primary, 23% in metastatic) in detecting gene amplification.
**Conclusions:** Only a small fraction of EGFR-IHC positive CRCs was associated with gene amplification, and the intensity of IHC staining does not always predict gene amplification. Whether EGFR gene amplification is more predictive of response to cetuximab than IHC is to be determined.

Table. IHC and CISH detection of EGFR in primary and metastatic CRCs.

| IHC | | CISH | | | Total |
|---|---|---|---|---|---|
| | | No amplification | low amplification | high amplification | |
| Primary CRC | 0 | 18 | 0 | 0 | 18 |
| | 1+ | 36 | 4 | 0 | 40 |
| | 2+ | 40 | 5 | 0 | 45 |
| | 3+ | 14 | 4 | 2 | 20 |
| Total | | 108 | 13 | 2 | 123 |
| Metastatic CRC | 0 | 5 | 0 | 0 | 5 |
| | 1+ | 10 | 0 | 0 | 10 |
| | 2+ | 7 | 1 | 0 | 8 |
| | 3+ | 0 | 0 | 1 | 1 |
| Total | | 22 | 1 | 1 | 24 |

### 541 Muscularis Mucosae Reduplication ("Ski Track Sign") Favors Ulcerative Colitis in the Differential Diagnosis of IBD

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**Background:** Endoscopic sampling of colorectal mucosa is a routine part of the diagnostic work-up of patients with ulcerative and Crohn's colitis (UC and CC, respectively). Nonetheless, distinction between the two disorders based on mucosal biopsy morphology alone is usually not achieved unless certain Crohn's-specific mucosal features, such as granulomas, focality of inflammation and segmental distribution, are identified. We have noted that colonic specimens from patients with UC are frequently characterized by reduplication of the muscularis mucosae (MM) resulting in a pair of parallel ski track-like muscular bands. We assessed the diagnostic value of this change with respect to the differential diagnosis of IBD, i.e., as a potential specific feature of UC.
**Design:** All available archived slides from 104 colectomy specimens were randomly selected from each of the following diagnostic categories and assessed for MM reduplication. They comprised 41 cases of UC, including 14 resected for indications of disease activity and 27 for long-standing disease with dysplasia or cancer, 50 of CC, including 19 with superficial (mucosal-based) CC (SCC), and 13 of other chronic inflammatory and mechanical colonic disorders (ischemic colitis, amebiasis, volvulus, diverticular disease). The mean number of slides per specimen was 27. Sections containing well-formed parallel bands of MM of roughly equal thickness and occupying at least one low-power field in length were recorded as positive for MM reduplication.
**Results:** MM reduplication was identified in 15 cases of UC (37%), 2 cases of conventional CC (6%) and 2 cases of SCC (11%), corresponding to a specificity for UC of 92% and sensitivity of 37% among the IBD cases. However, even among cases of conventional CC with mostly transmural inflammation, it was confined to small foci where the inflammation was mucosal-based. No MM reduplication was detected in the other disorders examined. The mean percentage of histologic sections affected per colectomy in UC was 9%. MM reduplication in UC occurred independently of anatomical site, disease duration and activity at colectomy except when the mucosa was extensively effaced by fulminant colitis.
**Conclusions:** MM reduplication as specified herein is specific to mucosal-based IBD. Given the low prevalence of superficial CC, the "ski track sign" may be useful in the diagnosis of UC in endoscopic biopsies that sample the muscularis mucosae.

### 542 The Diagnostic Value of Pancreatic-Duodenal Homeobox 1 (PDX-1) in Separating Metastatic Carcinoma of Unknown Origin

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**Background:** Pancreatic-duodenal homeobox 1 (PDX-1) is transcription factor in the ParaHox gene family and plays key roles in the genesis and development of the pancreas, duodenum and antrum. Mice with PDX-1 knockout show pancreatic agenesis and abnormal formation of the pylorus and duodenum. PDX-1 is also involved in the differentiation of endocrine cells in the gastric antrum. Preliminary data in our laboratory demonstrated that PDX-1 can be expressed in the adenocarcinomas of stomach, duodenum, bile duct and pancreas. However, the diagnostic value of PDX-1 expression in separating adenocarcinoma of the lung, breast, prostate and colorectum has not been investigated.
**Design:** A total 105 cases of adenocarcinomas including each 15 cases from lung, breast and prostate, 20 cases of colorectum, as well as 40 cases from gastro-esophageal junction, stomach, duodenum, bile duct and pancreas. The immunostaining for PDX1 was performed on an automated immunostainer using biotin-avidin-complex method with appropriate positive and negative controls. The statistical analysis was performed with chi-square method.
**Results:** Positive PDX-1 stain was counted when the nuclear stain was present.

| Carcinoma | GI tract, bile duct and pancreas | Lung | Breast | Colorectum | Prostate |
|---|---|---|---|---|---|
| PDX1 | 40% (16/40) | 0% (0/15) | 0% (0/15) | 15% (3/20) | 0% (0/15) |

**Conclusions:** 1), PDX-1is expressed in the adenocarcinomas of gastrointestinal tract, bile duct and pancreas, but not in the adenocarcinomas of the lung, breast and prostate. 2). PDX-1 should be included in a panel of immunohistochemical markers in the work-up of metastatic adenocarcinoma of unknown origin.

### 543 Genomic Gains on Chromosome 8q and Prognosis of Stage 2 Colonic Adenocarcinoma: An Array-Based Comparative Genomic Hybridization (A-CGH) and Fluorescence In Situ Hybridization (FISH) Study

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**Background:** Approximately 30% of stage 2 colonic adenocarcinoma (CAC) recur or metastasize within 3-5 years after surgery. Identification of tumors prone to such aggressive course would be useful for determining prognosis and customizing adjuvant therapy. We have previously detected gain of genomic material on chromosome 8q in such aggressive tumors by low-density A-CGH. Here, we studied genomic imbalances in stage 2 CAC from patients with long-term survival data using a high-density bacterial artificial chromosome (BAC) array.

**Design:** 17 frozen samples of uniformly staged, moderately differentiated CAC (T3N0M0, 12+ analyzed negative lymph nodes) treated by surgery alone were selected. Genomic changes in tumors with 10+ years recurrence free survival (non-progressors; n=10) were compared to those with recurrence/metastasis within 5 years from surgery (progressors; n=7). Whole genomic DNA from samples and reference DNA were labeled by random priming using Cy3 and Cy5, and co-hybridized to microarrays containing triplicate spots of approximately 6000 RPCI-11 genomic BAC clones (Roswell Park Cancer Institute, Buffalo, NY). The fluorescence ratios and their log-transformed values were analyzed using circular binary segmentation, a 2.5 std. deviation cut-off method and a Wilcoxon rank-sum genome scan.

**Results:** The extent of alterations overall and genomic gains alone, were marginally higher in progressors compared to non-progressors (p=0.05 and 0.06). While a spectrum of genomic abnormalities was detected in both groups, genomic gains at chromosome 8q occurred typically in the poor-outcome tumors compared to those with favorable outcome. The gains spanned most of the 8q arm and FISH performed on tumor touch preparations showed 8q24 (c-myc) region gains in all progressors but none of the non-progressors from this series.

**Conclusions:** Array-CGH can detect distinct genomic changes among stage 2 CAC. The presence of genomic gains at 8q appears to be a common feature of tumors with poor outcome and may distinguish them from non-progressing tumors. Assessment of the clinical value of such changes in a greater cohort requires further study.

### 544 Epidermal Growth Factor Receptor Expression in Colorectal Carcinoma - A Tissue Microarray-Based Study

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**Background:** Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase whose activation initiates an intracellular pathway that regulates cellular differentiation, proliferation, and angiogenesis. Overexpression of EGFR has been demonstrated in a variety of carcinomas, including colorectal cancers. We studied EGFR expression in colorectal carcinomas and compared EGFR immunostaining with the tumor microsatellite instability status, size, differentiation, and site in the colon, as well as patient age and gender, and the presence of lymph node metastases at the time of diagnosis.

**Design:** Sixty-nine cases of primary colorectal carcinoma were selected from the archival files. Patients who received chemotherapy or radiation for colorectal cancer prior to resection were excluded from the study. The carcinomas were graded as well, moderately, or poorly differentiated according to the WHO criteria. Additionally, patient age, gender, tumor microsatellite instability status, size and site within the colon, and the presence of lymph node metastases at initial diagnosis, were noted for each case. Tissue microarrays were created with 2.0 mm diameter cores (two per case) that were selected from invasive areas of the tumors. Slides were cut and immunostained within a week with EGFR antibody (DakoCytomation, Carpinteria, CA). Two observers assessed intensity (0, 1+, 2+) of the membranous staining. Greater than 1% membranous staining was considered positive, and controls stained appropriately.

**Results:** 47 of 69 (68%) cases showed EGFR expression. Chi-square tests of homogeneity were used to compare the intensity of staining with various parameters of the patients and tumors. Fisher's exact test was used when cell frequencies were small. No statistically significant correlations were found between EGFR staining and tumor size, location, differentiation, or microsatellite status. Additionally, there was no association with patient age or gender, or the lymph node status.

**Conclusions:** Previous studies have shown that patients with high expression of EGFR have increased risk of relapse and decreased survival. Therapies targeting the EGFR pathway are currently in use or under investigation for the treatment of colorectal carcinoma. In our study, EGFR did not correlate with any of the patient demographics or the tumor characteristics, including lymph node metastases at the time of diagnosis.

### 545 Morphological Parameters Are Useful in Distinguishing Barrett's Esophagus from Carditis with Intestinal Metaplasia

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**Background:** Intestinalized epithelium in mucosal biopsies from the gastroesophageal junction (GEJ) may represent either Barretts esophagus (BE) or gastric carditis with intestinal metaplasia (CIM). Differentiating these two conditions is important with regard to treatment and prognosis. The value of immunohistochemical studies, such as CK7/20, in this differential is controversial and fraught with technical limitations. The aim of this study was to evaluate a wide variety of objective morphological parameters in an effort to determine if any might be useful in distinguishing BE from CIM.

**Design:** Routinely processed mucosal biopsies from 20 patients with BE (mean age; 59.7 yrs, M/F ratio:13/7, mean BE length; 5.5cm) and 20 with CIM (mean age; 66.4 yrs, M/F ratio: 7/13) but without endoscopic evidence of BE were systematically evaluated in a blinded fashion for the presence of a wide variety of histological features, such as squamous mucosa overlying crypts with IM, hybrid glands (IM confined only to the superficial aspects of mucus type glands), esophageal glands/ducts, extent of IM in the crypts, incomplete type of IM, multilayered epithelium, crypt disarray and atrophy, and configuration of the surface epithelium (flat, tufted, serrated or villiform).

**Results:** The presence of the following eight parameters showed statistically significant differences between the two patient groups (BE vs. CIM): squamous mucosa overlying crypts with IM (57% vs 0; p=0.01), hybrid glands (40% vs 0; p=0.01), esophageal glands/ducts (30% vs 0; p=0.02), IM involving >50% of crypts (60% vs 10%; p=0.002), incomplete type of IM (100% vs 50%; p<0.001), multilayered epithelium (70% vs 15%; p=0.001), crypt disarray (85% vs 50%; p=0.04) and atrophy (60% vs 20%; p=0.02). None of the other parameters were significantly different between the two groups. The probability that a biopsy represented BE increased with an increasing number of features present. For instance, >4 or >5 features were present in 19/20 (95%) and 11/20 (55%) of BE cases, respectively, compared to 1/20 (5%) and 0/20 of CIM cases, respectively (p<0.05).

**Conclusions:** A number of morphologic features, particularly when present in conjunction, are highly specific for the distinction of BE from CIM on mucosal biopsy analysis.

### 546 Extent of Low Grade Dysplasia Is a Significant Risk Factor for Cancer in Barrett's Esophagus

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**Background:** Dysplasia is a well-known risk factor for progression to cancer in Barrett's esophagus (BE). Although one previous study showed that the extent of high-grade dysplasia (HGD) was a significant risk factor for progression to cancer, the prognostic significance of the extent of low-grade dysplasia (LGD) as a risk factor has never been tested. The aim of this study was to evaluate the significance of the extent of LGD as a risk factor for cancer in a well-defined cohort of high risk BE patients who have been followed with a standardized jumbo forceps surveillance protocol, which includes 4 quadrant biopsies every 1-2 cm of BE.

**Design:** 1,488 routinely processed BE biopsies from 69 high risk BE patients (M/F ratio: 60/9, mean age: 61 yrs (range: 22-81), mean BE length: 6.8 cm (range: 0-19), 28 of whom eventually developed adenocarcinoma, were evaluated for a variety of pathologic features including the total number (No.) of crypts and the No. of crypts with LGD and the results were correlated with the clinical features, such as length of BE, and outcome (invasive cancer versus no cancer). Over 32,000 crypts were counted. The mean follow-up was 52 mths (range: 1-196). The mean number of biopsies per patient was 22 (range: 2-53) and the mean number of crypts per biopsy was 22 (range: 10-70). Statistics was performed using a COX regression model to account for follow-up intervals and censored data.

**Results:** Of the 41 non-cancer patients, 18 (44%), 0 (0%), 9 (22%) and 14 (34%) had a diagnosis of negative (neg), indefinite (indef), LGD and HGD, respectively, whereas in the 28 patients who ultimately developed invasive cancer, 6 (21%), 0 (0%), 5 (18%) and 17 (61%) had neg, indef, LGD and HGD in their pre-cancer surveillance biopsies. However, in initial surveillance biopsies, both the presence (p=0.006) and the extent of crypts with LGD was highly associated with subsequent development of invasive cancer. The mean No. of crypts with LGD in patients who developed invasive cancer was 5.8 compared to 2.6 in the non cancer patients (p<0.0001; *Chi-square* test for relative risk in Cox model).

**Conclusions:** The extent of LGD is a strong risk factor for the development of invasive cancer in BE. Further studies on a larger number of patients with longer follow-up will be done to confirm these findings.

### 547 Diagnostic Concordance and Role of Size and Site in the Identification of Sessile Serrated Adenomas

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**Background:** Sessile serrated adenomas (SSA) are distinguished from hyperplastic polyps (HP) by disorderly maturation or abnormal proliferation. The majority of SSA are large and right-sided. SSA may be precursors of microsatellite unstable colorectal cancer. However, consistent application of diagnostic criteria of SSA can be difficult. To circumvent this problem, it has been suggested that all large right-sided polyps with serrated features should be designated as SSA. We evaluated the presence of morphologic features of SSA in a series of serrated polyps, and correlated these features with size and site. We also determined the concordance of diagnosis among 3 gastrointestinal pathologists.

**Design:** We reviewed 146 colorectal polyps (68 right, 78 left) previously diagnosed as HP. Serrated adenomas were excluded. Polyps with disorderly maturation or abnormal proliferation were designated SSA. Disorderly maturation was defined as architectural atypia (crypt branching, horizontal crypts, deep extensive serrations), dystrophic goblet cells and nuclear atypia close to the surface, and abnormal proliferation as asymmetric proliferative zone and mitoses in upper half of crypts. Polyps that could not be classified were designated as intermediate (IN). SSA were correlated with size and site; polyps>=0.8 cm were designated as large. Three gastrointestinal pathologists reviewed 88 of the 146 cases to judge concordance of diagnosis using criteria agreed upon before slide review.

**Results:** Right (n=68/146): 9/40 (23%) large polyps were SSA, 26 (65%) were HP and 5 (12%) were IN. 2/28 (7%) small polyps were SSA and 26 (93%) were HP.

Left (n=78/146): 4/44 (9%) large polyps were SSA, 36 (82%) were HP and 4 (9%) were IN. 31/34 (91%) small polyps were HP, none were SSA and 3 (9%) were IN.

Diagnostic concordance (n=88): There was complete agreement among 3 pathologists in 77/88 (88%) cases and among 2 pathologists in 85/88 (97%) cases. On the right, complete concordance was seen in 36/40 (90%) polyps (9 SSA, 26 HP, 1 IN). On the left, complete agreement was seen in 41/48 (85.4%) cases (4 SSA, 37 HP).

**Conclusions:** The classification of serrated polyps into SSA and HP has high concordance (88%) when previously established morphologic criteria are strictly applied. SSA are more common among large (23% right, 9% left) than small polyps (7% right, 0 left). Since a majority of large right-sided polyps are HP (65%) and a significant number of large left-sided polyps are SSA (9%), classification of serrated colorectal polyps based on size and site alone is not reliable.

### 548 Overexpression of Phosphorylated Histone H3 Is an Independent Prognostic Indicator in Gastric Cancer Patients

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**Background:** So far Ki-67 immunostaining has been commonly used for assessing cell proliferation, however, previous reports on the evaluation of prognostic indicator for Ki-67 immunostaining have revealed discordant results in gastric cancer patients. Recently, antibodies for phosphorylated histone H3 have been employed to identify cells during mitotic phase because of its precise overexpression in mitosis. We assumed phosphorylated histone H3 overexpression to be a good prognostic indicator for gastric cancer patients and investigated immunohistochemically phosphorylated histone H3 overexpression comparing with Ki-67 overexpression in gastric cancer samples.

**Design:** 112 surgically resected primary gastric cancer cases were selected and histologically categorized in accordance with WHO, UICC, Lauren classification. Immunohistochemistry was done by polyclonal rabbit anti-Histone H3 antigen, phospho-specific ($Ser^{10}$), antibody (dilution; 1:50; CALBIOCHEM, Darmstadt, Germany) and monoclonal mouse anti-human Ki-67 antigen antibody (dilution; 1:50; DAKO Cytomation, Copenhagen, Denmark). Statistical analyses were performed using the program StatView J5.0 for Windows.

**Results:** Over and low expressions for phosphorylated histone H3 and Ki-67 were determined by the border of 75 percentile, 0.9 and 26, respectively, on the basis of immunohistochemical results. No correlation was found between phosphorylated histone H3 and Ki-67 ($P$=0.25). Correlations between phosphorylated histone H3 overexpression and clinico-pathological variables were noted for histological type, Lauren, and lymph node metastasis. With regard to Ki-67 overexpression, no correlation was evident with any of the clinico-pathological variables. By the Kaplan-Meier method with log-rank test, cases overexpressing phosphorylated histone H3 showed a poorer prognosis in comparison with low expression cases ($P$<0.01). On the contrary, Ki-67 expression did not influence prognosis ($P$=0.18). Multivariate analyses indicated that phosphorylated histone H3 overexpression is an independent prognostic factor together with lymphatic invasion and venous invasion.

**Conclusions:** It seems likely that phosphorylated histone H3 plays an important role in the progression of gastric cancer and its immunohistochemical investigation is useful for the prediction of gastric cancer patients' prognosis, superior to Ki-67.

### 549 Gastric Carcinoid Tumors: The Pathological Spectrum of an Immune Conundrum

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**Background:** Most gastric carcinoid tumors (GCT) (type-I) occur in association with achlorhydria, hypergastrinemia, and chronic atrophic gastritis (CAG) or pernicious anemia (PA). These tumors exhibit low grade cytology and are confined to the mucosa or submucosa. Type-II lesions occur in conjunction with MEN-I while sporadic GCTs (Type-III) exhibit little relationship with CAG, immune phenomena or hypergastrinemia. A subset of type-I GCTs may be associated with as yet uncharacterized autoimmune phenomena other than PA, and they require further evaluation and management since their prognosis is quite different from Type-III lesions.

**Design:** We analyzed retrospectively 64 cases of GCT and compared their clinical and pathological features.

**Results:** 42/65 tumors (65%) demonstrated CAG with and without PA (Table). 27 patients with PA exhibited hypergastrinemia. The majority (85%) maintained an indolent course except one developed lymph node metastasis and 3 (11%) developed fundic adenocarcinomas. Neither the pathological features nor the gastrin level predicted metastases. 15 Type-I tumors were associated with gastritis without PA and had elevated (2-40 fold) gastrin levels. 33% of these patients exhibited other autoimmune conditions (diabetes, autoimmune thyroiditis, scleroderma); these conditions also co-existed in 30% of the PA patients.

18/23 sporadic cases were located in the fundus and 5 in the antrum or pylorus. Most were clinically covert without hormone-related symptoms. In contrast to type-I GCTs, only one case had autoimmune-related condition. Histologically, type-III GCTs exhibited a more infiltrative pattern, with high grade cytologic features. Metastases occurred in 70% cases.

**Conclusions:** 33% of non-PA type-I GCTs are associated with other autoimmune conditions. PA-associated GCTs are at risk for adenocarcinomas. In contrast, sporadic GCTs lack autoimmune associations and are more clinically aggressive. Appropriate clinical management of immune diseases warrants consideration of the co-existence and pathological consequences of GCTs.

Table. Clinical and Pathological Features of GCTs

| | Case Numbers | Gastrin (pg/ml) | Other Immune Conditions | Co-existing AdenoCa | Mitosis #/ 50HPF | Distant Metastasis | LN Metastasis |
|---|---|---|---|---|---|---|---|
| Total | 65 | | 14/65 (22%) | 3 | | 14/65 (21%) | 6/65 (9%) |
| Type I | 42 (65%) | | 13/42 (31%) | 3 | <1 (0-6) | 2/42 (5%) | 1/42 (2%) |
| Type I (PA) | 27/42 (65%) | 226-2193 | 8/27 (30%) | 3/27 (11%) | | 0 | 1/27 (4%) |
| Type I (Non-PA) | 15/42 (35%) | 52-4030 | 5/15 (33%) | 0 | | 2/15 (13%) | 0 |
| Type III | 23/65 (35%) | Normal | 1/23 (4%) | 0 | 15 (0-55) | 11/23 (48%) | 5/23 (22%) |

### 550 Defective Mismatch Repair Proteins in Adenomas and Adenocarcinomas of the Small Bowel: An Immunohistochemical Analysis

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**Background:** Carcinomas and adenomas of the small bowel are rare, but the risk of developing these lesions is increased in a variety of conditions, including Hereditary Non-Polyposis Colorectal Cancer (HNPCC). HNPCC is associated with mutations in genes involved in mismatch repair mechanisms, most commonly hMLH1 and hMSH2. Microsatellite instability has been associated with right-sided colonic neoplasms. We investigated DNA mismatch repair defects in neoplasms of the small bowel because both the major portion of the small bowel and right side of colon embryologically derive from the midgut.

**Design:** In a retrospective study of archival samples from our institution from the past 22 years, we analyzed the immunohistochemical expression of hMLH1 and hMSH2 in 27 small bowel neoplasms, including 14 adenomas and 13 adenocarcinomas. The majority (22) were located in the duodenum, including 12 ampullary neoplasms. The remainders were located in the ileum (3), jejunum (1), and unspecified location in the small bowel (1). Patients in this study ranged in age from 17 to 91 years.

**Results:** Of 14 small bowel adenomas, 1 (7%) in a 53 year-old female (ampullary) showed a loss of staining for hMSH2, whereas the remainder (93%) showed normal reactivity for hMLH1 and hMSH2. Of the 13 adenocarcinomas, 1 (8%) in a 65 year-old female (duodenal) lost expression of hMSH2, whereas the remainder (92%) stained both hMLH1 and hMSH2.

**Conclusions:** In small bowel neoplasms from an unselected population, loss of immunostaining for a DNA mismatch repair enzyme (hMSH2) was found in only 7% of adenomas and 8% of adenocarcinomas. Mismatch repair defects may result from a germline mismatch repair gene mutation, or from somatic mismatch repair gene inactivation. In general, loss of staining for the hMSH2 protein is almost always due to germline mutation in the hMSH2 gene, while loss of expression of hMLH1 is frequently associated with somatic hypermethylation. The rates of mismatch repair defects seen in small bowel neoplasms in this study are somewhat lower than the estimated rate (10 – 15%) of inactivation of mismatch repair mechanisms in unselected colorectal carcinomas. While it is reasonable to test small bowel neoplasms for the presence of hMLH1 and hMSH2, patient age and family history remain essential elements in screening for hereditary carcinoma syndromes.

### 551 Influence of Demographic and Specimen Related Parameters on EGFR Immunostaining in Colorectal Adenocarcinoma: A Study of 484 Cases

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**Background:** The EGFR pharmDx antibody (DakoCytomation) is currently used in the determination of patients with colorectal carcinomas eligible for treatment with cetuximab. While the critiera for scoring tumor positivity have been established, the relationship of the EGFR scoring results to demographic factors, as well as factors relating to the nature of the specimen (e.g., tumor grade, primary vs. metastatic tumor, specimen size) are unknown.

**Design:** Immunohistochemical analysis of EGFR expression was performed on 500 slides (484 patients) from 99 institutions. The patients had either primary (459 slides) or metastatic (41 slides) colorectal carcinomas. Deparaffinized sections were immunostained with the DakoCytomation EGFR pharmDx kit according to the supplied protocol, and the tumors were scored by a single pathologist, using the FDA-approved criteria. Statistical analysis was then performed to determine the relationship of the EGFR pharmDx score to a number of demographic factors, including patient age and sex, as well as specimen-related parameters such as tumor grade, specimen type submitted for analysis, and primary versus metastatic tumor.

**Results:** The overall rate of EGFR positivity was 370/499 (74.15%); EGFR 1+ was noted in 44.09%, 2+ in 22.24%, and 3+ in 7.82%. This positivity rate was significantly higher in sections from resected specimens (76.19%) than in biopsies from either primary tumor (66.25%) or metastasis (68.29%) ($p < 0.02$). EGFR 1+ was observed in 52.5% of the biopsies from metastases, while 2+ and 3+ were seen each in 9.76% of the cases. Poorly differentiated tumors had a higher incidence of positivity to EGFR (88.10%) than well and moderately differentiated tumors (72.87%). Concordant EGFR expression in both the primary tumor and metastasis was noted in 7/12 patients examined (58.33%).

**Conclusions:** A higher frequency of EGFR positivity was found in resected specimens as compared to biopsies, suggesting that sampling error may contribute to the probability of changing scores from negative to 1+. In addition, high-grade tumors are more likely to express EGFR. Other factors such as the sex and age of patients, or examination of primary versus metastatic tumor do not appear to influence the EGFR scores. These factors should therefore be considered in determining the expected or appropriate EGFR positivity rate using the pharmDx antibody kit.

### 552 Wilm's Tumor Gene Protein (WT-1) and Calretinin (Cal) Immunoreactivity in Gastrointestinal Stromal Tumor (GIST)

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**Background:** GIST exhibits a phenotype similar to that of the interstitial cell of Cajal (ICC). The apparent relationship between GIST and ICC is predicated in part on shared expression of proteins, most notable among them c-kit. Identification of novel protein expression patterns that are similarly shared by ICC and GIST may help to understand the underlying relationship between the normal cell population and its neoplastic counterpart. Recently, it has become known that WT1 and Cal are detected in peripheral nerve cells. The goal of the current study is to evaluate the diagnostic utility of WT-1 and Cal in GIST.

**Design:** Formalin-fixed, paraffin-embedded sections were prepared from 131 primary GIST cases (stomach; 92 cases, duodenum; 10 cases, jejunum; 9 cases, ileum; 2 cases, rectum; 13 cases, mesentery; 4 cases, vagina; 1 case, respectively), 14 secondary GIST cases (5 cases; liver, 5 cases; peritoneum, 4 cases; other sites) and 12 other gastrointestinal soft tissue tumor cases, including 8 leiomyoma and 3 gastrointestinal autonomic nerve tumor (GANT). Sections were serially sectioned and stained with HE and WT-1, c-kit, smooth muscle actin (SMA), S-100, and Cal, respectively.

**Results:** WT-1, Cal and c-kit were positive for normal ICC. WT-1 and calretinin showed granular cytoplasmic or subplasmalemmal linear staining pattern. Immunoreactivity was scored from 0 to 3+(0=no or faint staining <10% in tumor cells, 1+=faint staining in >10% of tumor cells, 2+=moderate or strong staining in <33% of tumor cells, 3+=moderate or strong staining in >67% of tumor cells). The immunohistological results of GIST for WT-1 are summarized in the following table.All c-kit negative GIST cases were 3+ positive for WT-1. All GANT cases were 3+ positive for WT-1 and negative for c-kit. One of them was 1+ positive and two of them were negative for Cal. All leiomyoma cases are both negative for WT-1, Cal, and c-kit..

**Conclusions:** The predominantly cytoplasmic pattern of WT-1 immunoreactivity in ICC is recapitulated in GIST. These results strengthen the presumptive relationship between ICC and GIST. WT-1 could be a novel immunohistochemical marker that is both sensitive and specific for GIST. Loss of Cal expression may play an important role in tumorigenesis of GIST.

| | Primary | | Primary | | Secondary | |
|---|---|---|---|---|---|---|
| Intensity | WT-1 | Calretinin | C-kit | WT-1 | Calretinin | C-kit |
| 0 | 0 | 5 | 122 | 3 | 2 | 13 |
| 1+ | 2 | 1 | 1 | 0 | 0 | 1 |
| 2+ | 3 | 3 | 6 | 0 | 0 | 0 |
| 3+ | 126 | 122 | 2 | 11 | 12 | 0 |

### 553 Impact of Bayes Theorem and Poisson Paradigm on Outcomes in Node Negative Colorectal Cancer Patients after Surgical Resection

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**Background:** It has been demonstrated that node-negative colorectal cancer patients have a worse outcome if fewer than 7 lymph nodes are examined from their surgical specimens. This result agrees with the Poisson paradigm, which suggests that the probability of finding a positive node increases with the total number of nodes examined. Using the Poisson model, Bayes theorem provides a way to estimate the probability of metastasis to a lymph node, when in fact no metastasis has been observed. We sought to explore whether these models can help identify a subset of patients with a worse outcome despite a node-negative resection.

**Design:** The study comprised 297 patients with resected colorectal carcinomas. 122 had positive lymph nodes, and 175 did not. The mean follow-up time of those with negative nodes was 43 months. We calculated the Bayes probability of metastasis given that none had been observed as:

Bayes = 1 divided by [1 + (prior odds of no metastasis)/Poisson probability]

The prior odds were estimated from the odds of no metastases in all of the patients studied. The Poisson probability used was exp(- $\alpha$ * n), which gives the probability of observing negative nodes when n is the total number examined. $\alpha$ is the Poisson parameter, which was modeled using the data of the 122 patients with positive nodes. The main results comprised a survival analysis of the 175 with negative nodes.

**Results:** In the 175 patients with negative nodes, the calculated Bayes probability of undiscovered lymph node metastasis averaged 0.10 (range 4.3 x 10-8 to 0.42). Cox model analysis showed that the Bayes probability was significantly related to subsequent survival ($p = 0.0002$); whereas, T stage provided no further prognostic information ($p > 0.1$). Most of the impact on survival was due to those with a Bayes probability greater than 0.15, and a Kaplan-Meier survival plot further demonstrates this cutpoint.

**Conclusions:** The Bayes probability using the Poisson model can be calculated for any node negative patient with resected colorectal cancer and utilizes just the T stage of the tumor plus the number of examined nodes. When this calculated value exceeds 0.15, survival appears to be worse. Thus, the results suggest that this subset of patients may require adjuvant therapy.

### 554 Pleomorphic Gastrointestinal Stromal Tumors: Diagnostic and Therapeutic Implications

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**Background:** Gastrointestinal (GI) stromal tumor (GIST) is the most common mesenchymal tumor of the GI tract. Histologically, GISTs are almost invariably monomorphic in appearance. However, rare cases exhibit marked pleomorphism and this can cause considerable diagnostic confusion. This is important since misdiagnosis may lead to inappropriate therapy. In this study, we reviewed the clinical and pathologic features of 27 cases of pleomorphic GIST.

**Design:** 1250 cases of GIST were reviewed and 27 cases with pleomorphic cells visible at 10x and occupying at least one field were identified. The clinical and pathological features were recorded. Immunohistochemical studies with KIT (CD117), CD34, desmin, smooth muscle actin (SMA), S-100 protein, and pan-cytokeratin (AE1/AE3) were performed and *KIT* and *PDGFRA* mutation status were determined.

**Results:** Complete clinical features were available in 26 cases. There were 14 M/12F and patient age ranged from 25-88 (median 54). The lesions involved the stomach (16) small bowel (5), mesentery (1), retroperitoneum (1) and pelvis (1). Three cases were disseminated at presentation. Tumor size ranged from 2-34 cm (median 12 cm). Follow-up was available in 19 patients ranging from 1-62 months (median 9 months). 10 cases metastasized to the liver or disseminated. 8 of 17 patients were alive with disease and 2 patients died of disease at 1 month and 18 months respectively. Immunohistochemistry: KIT (22/26), CD34 (7/19), desmin (1/18), SMA (8/16), S-100 protein (2/18 - focal), pan-cytokeratin (1/12- focal). Activating *KIT* and *PDGFRA* mutations were seen in 13 and 6 cases respectively. Three cases were wild-type for both *KIT* and *PDGFRA.* Two of 4 KIT negative cases by immunohistochemistry harbored *PDGFRA* mutations.

**Conclusions:** Pleomorphic GISTs are very uncommon. They appear to exhibit clinicopathologic features similar to non-pleomorphic GISTs but may be more aggressive. *PDGFRA* mutations appear to be more common (27% in this series). It is important to recognize pleomorphic GISTs since many contain activating *KIT*mutations and thus, would likely respond to therapy with imatinib mesylate (Gleevec).

### 555 p16 and Ki67 Immunostaining Is a Useful Adjunct in the Assessment of Biopsies for HPV-Induced Anal Intraepithelial Neoplasia (AIN)

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**Background:** p16 is a tumor suppressor gene product, shown to be overexpressed in most cervical carcinomas and dysplasias induced by high risk human papilloma virus (HPV). HPV- associated squamous dysplasias and carcinomas are also found in the anal canal of homosexual/bisexual males. Significant inter- and intra-observer variation exists in the interpretation of anal intraepithelial neoplasia (AIN). This study was undertaken to determine if p16 and Ki67 immunohistochemical expression is useful in refining the diagnosis and grading of AIN.

**Design:** Seventy-one anal lesions from 49 patients were retrieved from the Pathology Department files. These included 14 cases with reactive changes, 9 hemorrhoids, 8 condylomas, 9 AIN I, 13 AIN II, 16 AIN III, and 2 invasive squamous carcinomas (SCC). Immunostaining for p16 and Ki67 was done by ABC immunoperoxidase. Positive and negative staining was correlated with H&E diagnosis. Slides were reviewed by two pathologists initially and discrepancies were resolved by a third pathologist.

**Results:** Both nuclear and cytoplasmic staining was considered as positive for p16 when present in >25% of cells. Two patterns of p16 staining were observed: (1) scattered positive cells distributed throughout the lesion; and (2) a band-like staining of all dysplastic cells. Ki67 was read as positive when present in >25% of nuclei. A band-like pattern of p16 immunoreactivity was seen in 22% AIN I, 86% AIN II and100% AIN III and SCC. Spotty positivity was observed in hemorrhoids, condylomas, AIN I and rarely in AIN II (see table). Ki67 positivity was present in none of the hemorrhoids, 21% cases with reactive changes, 75% condylomas, 55% AIN1, 85% AIN2, 94% AIN3, and 100% SCC.Concordance between H&E staining, and p16 and Ki67 immunostaining was observed in 73% of cases.

| H&E diagnosis | No. of cases | p16 staining (expressed as %) | | |
|---|---|---|---|---|
| | | Negative | Spotty | Band-like |
| Hemmorhoids | 9 | 78 | 11 | 11 |
| Reactive | 14 | 100 | 0 | 0 |
| Condyloma | 8 | 63 | 37 | 0 |
| AIN I | 9 | 56 | 22 | 22 |
| AIN II | 13 | 7 | 7 | 86 |
| AIN III | 16 | 0 | 0 | 100 |
| SCC | 2 | 0 | 0 | 100 |

**Conclusions:** When used together, and in conjuction with H&E staining, p16 and Ki67 immunoexpresssion is a useful adjunct in the diagnosis and grading of AIN. A band-like pattern of p16 positivity is a highly specific marker for high grade AIN.

### 556 Expression of IGFBP2 in Carcinoid Tumors and Pancreatic Endocrine Tumors

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**Background:** Insulin-like growth factor binding proteins (IGFBPs) comprise a family of proteins that bind and regulate the functions of insulin-like growth factors. Recent studies have shown that overexpression of IGFBP2 may contribute to the invasiveness and progression of human malignancies including glioma, ovarian and prostate cancers. IGFBP2 mRNA has been shown to be expressed in most of the pancreatic endocrine tumors (PETs) and carcinoid tumors of gastrointestinal tract, suggesting a possible role of IGFBP2 in the progression of these tumors. However, the expression pattern of IGFBP2 in neuroendocrine tumor has not been examined in detail.

**Design:** We evaluated the expression of IGFBP2 by immunohistochemistry in 42 neuroendocrine tumors including 15 PETs and 27 carcinoid tumors of small bowel (20 primary and 7 metastatic tumors). The IGFBP2 expression was evaluated semiquantitatively as percentage of tumor cells with positive cytoplasmic staining. The tumors were categorized into positive (cytoplasmic staining in ≥10% of tumor cells) or negative (no staining or cytoplasmic staining in <10% of tumor cells) and the results were correlated with the clinicopathologic features.

**Results:** IGFBP2 was expressed in 73% (11/15) of PETs, 55% (11/20) of primary carcinoid tumor of small bowel, and 29% (2/7) of metastatic carcinoid tumors. The average percentage of IGFBP2 positive tumor cells was significantly higher in PETs (64.7% ± 46.4%) than that in carcinoid tumors (25.0% ± 31.8%) (p=0.008). The staining pattern of IGFBP2 was different between the PETs and carcinoid tumors. Fifty-four percent (7/13) of the IGFBP2-positive carcinoid tumors had staining only at the periphery of the tumor. In contrast, 91% (10/11) of IGFBP2-positive PETs showed diffuse tumor cell staining (p=0.03).
**Conclusions:** Our study shows that IGFBP2 is frequently expressed in neuroendocrine tumors and there is a difference in the staining pattern of IGFBP2 between PETs and carcinoid tumors of the small bowel.

### 557 Detection of *Helicobacter Pylori* by Multiplex PCR: Comparison with Immunohistochemistry and CLOtest

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**Background:** *Helicobacter pylori* (HP) is associated with up to 95% of duodenal and gastric ulcers and with the development of gastric carcinoma and MALT lymphoma. There are a wide variety of tests available for detecting this important organism. The aim of this study is to compare the detection ability of a novel PCR assay with standard methods of immunohistochemistry (I) and CLOtest (C).
**Design:** Upper endoscopy was performed on 61 patients (30M,31F ages 18-94) for symptoms of dyspepsia. Biopsies of the antrum/body (S) and/or gastroesophageal junction (E) were submitted for routine histology. The degree of mononuclear and PMN inflammation was scored [none (1), mild (2),moderate (3), severe (4)] and the presence of HP was evaluated by immunohistochemistry (Novocastra). Separate paired biopsies of the (S) (n=57) and (E) (n=45) were evaluated by a novel one-step multiplex PCR assay examining 5 loci in the HP genome. Separate antrum biopsies were submitted for C and interpreted using standard methodology.
**Results:** 59% (36/61) of patients overall were PCR(+) in (S) and/or (E). Comparison with I and C is provided in Table 1. All (E) I(+) PCR(-) were (S) PCR(+). Table 2 lists the inflammation score in the various subgroups.
**Conclusions:** PCR accurately identifies HP detected by I and C. PCR detects the presence of HP in a significant number of cases in which the organism is not identified by these routine methods. I (-) C(-) PCR (+) biopsies show inflammation scores similar to I(-) C(-) PCR (-). In the (S) most I(-)C(-)PCR(+) are histologically normal.

Table 1. Summary of PCR, CLOtest and immunohistochemistry results

| | (S) PCR(+) | (S) PCR(-) | (E) PCR(+) | (E) PCR(-) |
|---|---|---|---|---|
| I(+) | 11 | 0 | 1 | 4 |
| I(-) | 18 | 28 | 14 | 26 |
| C(+) | 13 | 0 | Not done | Not done |
| C(-) | 19 | 27 | Not done | Not done |

Table 2. Correlation of inflammation score with PCR and I results

| | (S) PCR (+) I(+) | (S) PCR (-) I(-) | (S) PCR (+) I(-) | (E) PCR (+) I(+) | (E) PCR (-) I(-) | (E) PCR (+) I(-) | (E) PCR (-) I(+) |
|---|---|---|---|---|---|---|---|
| PMN | 36% | 100% | 94% | 100% | 96% | 100% | 50% |
| IS ≤2 | (4/11) | (28/28) | (17/18) | (1/1) | (25/26) | (14/14) | (2/4) |
| PMN | 64% | 0% | 6% | 0% | 4% | 0% | 50% |
| IS ≥3 | (7/11) | (0/28) | (1/18) | (0/1) | (1/26) | (0/14) | (2/4) |
| MN | 0% | 82% | 83% | 0% | 31% | 71% | 0% |
| IS ≤2 | (0/11) | (23/28) | (15/18) | (0/1) | (8/26) | (10/14) | (0/4) |
| MN | 100% | 18% | 17% | 100% | 69% | 29% | 100% |
| IS ≥3 | (11/11) | (5/28) | (3/18) | (1/1) | (18/26) | (4/14) | (4/4) |

PMN - Polymorphonuclear cells, MN - Mononuclear cells, IS - Inflammation score

### 558 Epidermal Growth Factor Receptor (EGFR) Expression in Colorectal Cancer and Clinical Outcome of Anti-EGFR Therapy

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**Background:** EGFR is a transmembrane cell surface receptor overexpressed in various epithelial tumors, including 25-77% colorectal carcinomas. ERBITUX™ (cetuximab) is a monoclonal antibody targeted against EGFR and has recently been approved for treating EGFR-expressing, metastatic colorectal cancer. ERBITUX™ binds specifically to EGFR and blocks certain growth factors from binding and subsequently signaling the cell to promote tumor cell growth, survival, and progression. Assessing EGFR expression level is important for patient management and identifying patients who would benefit from ERBITUX™ therapy.
**Design:** Fifty patients with ≥ stage II colorectal cancer were examined for EGFR protein expression by immunohistochemistry (IHC) (EGFR pharmDx™, Dakocytomation). EGFR expression was quantified using an automated ceullar imaging system (ACIS, Chromavision). Expression distribution and differences between primary and metastatic sites were examined. Simultaneously, copy number of the EGFR gene locus was studied. Fluorescence in situ hybridization (FISH) analyses were initiated using an EGFR locus specific probe for the EGFR locus on chromosome 7 at 7p12 (LSI® EGFR SpectrumOrange™/CEP® 7 SpectrumGreen™). FISH and IHC results were correlated. Select patients showing EGFR-positive tumors were candidates for ERBITUX™.
**Results:** Preliminary data show that 60% of patients studied with colorectal cancer have EGFR membrane and/or cytoplasmic expression by IHC. The immunoreactive tumor cells ranged from 10% to 75%. Patients with metastatic carcinoma had at least 50% cells with moderate to strong positivity at metastatic foci, while the primary sites showed 0-20% immunoreactive cells, suggesting a gain of EGFR overexpression mutation in metastasis. EGFR failed to show gene amplication by FISH. The average of EGFR gene copy number was statistically the same as that of control gene. Correlation of EGFR staining intensity with response rate, progression, and survival is ongoing.
**Conclusions:** EGFR is overexpressed in a large percentage of colorectal carcinomas and metastases appear to gain higher level of expression compared to the primary sites. The mechanisms could be transcription upregulation or other causes versus gene amplification, as illustrated by FISH. Quantitative IHC remains a valid method of measurement. However, since none of our cases with EGFR protein overexpression demonstrate gene amplification, other expression regulating mechanisms must be considered.

### 559 Gastric Graft vs. Host Disease Revisited: Does Proton Pump Inhibitor Therapy Affect Endoscopic Gastric Biopsy Interpretation?

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**Background:** Because graft vs. host disease (GvHD) is associated with significant morbidity and mortality, accurate diagnosis is important. To test the hypothesis that proton pump inhibitor (PPI) therapy may interfere with histologic evaluation of gastric GvHD by inducing apoptosis, we evaluated epithelial apoptotic body counts in antral and fundic biopsies from bone marrow transplant (BMT) recipients and control patients, both taking and not taking PPI's at the time of endoscopic biopsy.
**Design:** H&E stained slides of gastric biopsies from 130 patients (75 allogeneic BMT or stem cell transplant recipients with GvHD on clinical and histologic grounds, and 55 age-and sex-matched control non-BMT patients with histologically normal gastric biopsies) were reviewed. The groups were further stratified into patients taking (PPI+) and not taking PPI's (PPI-) at the time of biopsy. Apoptotic bodies (AB)/10 high power fields (hpf) were quantitified for each case. Mean apoptotic body counts were then calculated for each case group. Immunohistochemical stains for gastrin were performed on 31 antral control cases.
**Results:** In the PPI- groups, apoptosis was increased in biopsies from BMT patients, compared to controls, both in antral (p=0.002) and fundic mucosa (p=0.03). In PPI+ patients, there was significantly more apoptosis in the gastric body in BMT patients than in controls (p=0.01).

Apoptotic Index (AB/10 high power (40x) fields)

| Group | Antrum, + PPI | Antrum, no PPI | Body, + PPI | Body, no PPI |
|---|---|---|---|---|
| BMT | 2.4 (N=25) | 2.3 (N=29) | 3.5 (N=15) | 3.8 (N=6) |
| Control | 1.7 (N=18) | 0.3 (N=15) | 0.6 (N=14) | 0.1 (N=8) |

However, comparing antral biopsies from control and BMT PPI+ patients, there was no significant difference in AB quantitation (p=0.275). More apoptosis was seen in antral biopsies from PPI+ control patients when compared to PPI- control patients (p=0.009). Gastrin-positive cells were increased in antral biopsies from PPI+ patients (85/10hpf) compared to PPI- (48/10hpf). Increased numbers of gastrin positive cells correlated with higher apoptotic body counts.
**Conclusions:** PPI therapy is associated with increased apoptosis in antral biopsies, and may interfere with the evaluation of GvHD in antral biopsies. A similar increase in apoptosis was not seen in fundic biopsies, and thus our data support the preferential biopsy of the gastric fundus for diagnosis of upper GI GvHD. Increased antral apoptosis is also associated with increased numbers of gastrin-positive cells, a finding warranting further investigation.

### 560 Histologic Predictors of 5 Year Survival in 169 Patients with Right Sided Colon Cancer

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**Background:** Recent studies have identified a number of histologic parameters as important predictors of patient outcome in colon cancer [CC]. These findings have occurred in association with continued updates of WHO nomenclature and enhancements in TNM staging guidelines as well as understanding of the need for adequate lymph node dissection. To assess the relative importance of these separate concepts we performed an analysis of right-sided CC patients incorporating these guidelines to assess their impact on patient survival.
**Design:** Patients were identified using pathology and cancer center databases at our institution. Patients were excluded based on inadequate long-term follow-up, missing pathology slides and inadequate numbers of lymph nodes in resection specimens. This latter group was defined as < 12 in N0 specimens and <10 lymph nodes when only one lymph node was positive. All patients with synchronous/metachronous CC and those with small cell carcinoma were excluded. The histologic slides were reviewed without knowledge as to patient outcome.
**Results:** 166 patients were entered into this study. At 5 years 54% of the patients had died of CC. Mean age at diagnosis of patients who were alive at 5 years was 71.3 compared to 70.3 who died due to CC. Overall 24 patients were Stage I; 48 were Stage II; 44 were Stage III and 50 were Stage IV. 71% of CC were low grade. Univariate analysis using likelihood Chi-square identified stage, number of positive lymph nodes, tumor type, grade, angiolymphatic invasion, perineural invasion, desmoplasia, tumor border configuration, tumor budding, intra-epithelial lymphocytosis, Crohn's like infiltrate and purulent infiltrate as important predictors of patient outcome. By multivariate analysis [Odds Ratio (95%CI)] advanced stage [Stage III and IV] [4.91 (1.62,14.84)], increased number of positive lymph nodes [1.23 (1.04, 1.45)] and absence of Crohn's like infiltrate [0.11 (0.03, 0.42)] were associated with worse patient outcome. All 35 patients with 6 or more positive lymph nodes and 46 of 49 stage 4 patients died of their disease within 5 years.
**Conclusions:** Recent recommendations by UICC, WHO and CAP pertaining to CC characterization, including assessment of adequacy of lymph node dissections, allow for better correlation between CC pathology reports and features predictive of patient outcome.

### 561 Minimal Collagenous Colitis: Microscopic Colitis with Minimal Subsurface Collagen Is Appropriately Diagnosed as Collagenous Colitis

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**Background:** The microscopic colitides—lymphocytic and collagenous colitis (LC and CC)—are characterized by chronic watery diarrhea, normal colonoscopy, and chronic nondistorting colitis with surface lymphocytosis. They differ in their demographics, response to therapy, and by subsurface collagen deposition that characterizes collagenous colitis, and is usually evident on H&E sections. There are cases of microscopic colitis with a thin, but structurally abnormal layer of subsurface collagen, evident by trichrome stain, but not in H&E sections. This study evaluates such cases, referred to as minimal collagenous colitis (MCC), to determine whether the demographic features, response to therapy and histologic features are more like CC or LC.

**Design:** Colorectal biopsies from 191 consecutive patients originally diagnosed as LC and CC were evaluated for minimal subsurface collagen deposition that was only apparent after trichrome staining. Additional histologic features were evaluated, including lamina propria eosinophilia, crypt lymphocytosis, and Paneth cell metaplasia. Treatment and clinical outcomes were assessed in 145 patients.

**Results:** 94 cases were classified as CC, 64 as LC, and 43 as MCC. Lamina propria eosinophilia and Paneth cell metaplasia are more common in CC than LC (79% vs 34%, and 58% vs 8%); whereas crypt lymphocytosis is more prominent in LC than CC (83% vs 18%). In MCC these histologic parameters were intermediate compared to CC and LC: 63% lamina propria eosinophilia, 26% Paneth cell metaplasia, and 63% crypt lymphocytosis. As expected, patients with LC were more likely to need no more than simple anti-diarrheal drugs (90%) than CC (62%); whereas, 33% of CC and only 8% of LC required steroid therapy. The MCC patients needed no more than anti-diarrheals in 66%, and required steroid therapy in 25%. Statistically, MCC was more like CC than LC in terms of those needing only simple anti-diarrheas and those requiring steroids. None of the additional histologic features evaluated was associated with therapeutic response..

**Conclusions:** Although histologic features of MCC are intermediate between CC and LC, the similarity to CC in terms of prognosis suggests that cases of microscopic colitis with minimal subsurface collagen are appropriately diagnosed as CC. We recommend that all cases thought to be LC be evaluated by trichrome stain to exclude MCC.

### 562 Contrasting Patterns of Activating Mutations of RAS-RAF-MAP-Kinase Signaling Pathway and CpG Island Methylation Phenotype (CIMP) in Typical and Serrated Colorectal Adenomas

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**Background:** Activating mutations of the RAS-RAF-MAP kinase pathway have been shown to represent early or instigating mutations in hyperplastic polyps, while CpG island methylation has been linked to later progression to serrated adenoma. In this study we compared these molecular patterns in serrated (SA) and typical (TA) adenomas.

**Design:** Paraffin sections from a sample of 25 SAs and 54 TAs were assayed for mutations at exon 15, codon 599 of BRAF using an allele-specific PCR (AS-PCR) and confirmed with direct sequencing of exon 11 and 15. Genomic sequencing was used to assay KRAS codon 12, 13, 59,60 and 61 mutations and methylation specific PCR to assay CIMP status using 5 markers, hMLH1, MGMT, MINT1,MINT2 and p16.

**Results:** BRAF and KRAS mutations were mutually exclusive findings in 15(60.0%) and 7(28.0%) of SAs respectively. Among TAs no cases had BRAF mutations and 6 (11%) had KRAS mutations but only in larger polyps (> 1cm) [p=.004]. CpG island methylation involving 2 or more genes (CIMP-H) was present in 80% of SAs compared to 13% of TAs [p<.001] and no TA had more than 3 markers positive compared to 28% of SAs [p<.001]. CIMP-H was significantly associated with larger size in TAs [p=.007] but independent of size in SAs. MGMT was methylated in 26% of TAs compared to 40% of SAs and correlated significantly with the presence of KRAS mutations in TAs [p= .001] but not in SAs.

**Conclusions:** SAs and TAs showed contrasting molecular profiles that offer insights into their molecular pathogenesis, and the relevance of the sequence/order of the molecular changes to adenoma phenotype.

### 563 Microvillus Inclusion Disease: Morphology, Organ Involvement, Biomarkers and Ultrastructure

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**Background:** Microvillus inclusion disease (MVID) is a rare congenital disease, producing intractable secretory diarrhea in early infancy. It is characterized by diffuse intestinal villous atrophy with no inflammatory reaction. Ultrastructural identification of apical microvillus inclusions in surface enterocytes is diagnostic. However, there is difficulty in the diagnosis of MVID due to the existence of variants (eg, microvillus dystrophy), possible disease resolution (one case report), and tissue orientation for electron microscopy (EM). We analyzed 4 cases of MVID from a single institution. The morphologic features, distribution of lesions, biomarkers, and complementary ultrastructural characteristics were studied.

**Design:** Four cases of MVID were collected at a large referral institution from 2 boys and two girls. One case was diagnosed at 4 months; the patient was followed for 10 years. All 4 patients had small bowel biopsies for review and 2 had colon and stomach biopsies. All intestinal biopsy specimens were analyzed histologically, histochemically (for PAS/AB and alkaline phosphatase), immunohistochemically (for CD10 and polyclonal CEA) and ultrastructurally.

**Results:** Ultrastructurally, apical microvillus inclusions in surface enterocytes in duodenal biopsies were identified in 3/4 cases, while one case had variant morphology (microvillus dystrophy). Tissue orientation for EM was crucial for identification of inclusions in apical enterocytes. Morphologically, a bubbly vacuolated appearance of the apical cytoplasm with extensive or patchy absence of the brush border with occasional cytoplasmic inclusions were observed in the enterocytes. Some of these changes vaguely resembled gastric mucin cell metaplasia. Architecturally, villous blunting with either crypt hypoplasia or hyperplasia and absence of inflammation were common findings. The crypts showed increased apoptosis and proliferation.The epithelial changes were also found in colon biopsies. PAS, alkaline phosphatase, CD10 or p-CEA showed a bright apical cytoplasmic blush, which correlated ultrastructurally with apical granules with inclusions of variable electron density in all cases. These stains also highlighted the targetoid inclusions in 3 of 4 cases, but not in the case of microvillus dystrophy.

**Conclusions:** Besides EM identification of inclusions, light microscopic morphological features together with the biomarker-highlighted apical cytoplasmic blush and inclusions are unique and diagnostic of MVID. The changes may be found in both the small bowel and colon.

### 564 Alterations in Das-1 and CG-3 Expression in Colonic Mucosal Biopsies Help Distinguish Ulcerative Colitis from Crohn's Disease

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**Background:** The distinction between ulcerative colitis (UC) and Crohn's disease (CD) is often difficult clinically and pathologically, particularly upon colonic mucosal biopsy analysis. Das-1 and CG-3 are monoclonal antibodies directed against an unidentified colonic epithelial protein and tropomyosin isoform 5, respectively, both of which are constitutively expressed cytoplasmic proteins previously shown to be altered in the UC colon. The aim of this study was to evaluate the pattern of reactivity and diagnostic utility of Das-1 and CG-3 in discriminating between UC and CD.

**Design:** One colonic biopsy specimen from each of 20 patients with clinically and pathologically confirmed UC (n=10, M/F ratio: 3/7, mean age: 38.3 yrs) or CD (n=10, M/F ratio: 5/5, age: 55.3 yrs), and 10 patients with no colonic pathology (M/F ratio: 3/7, mean age: 46.4 yrs) were stained for Das-1 and CG-3 by the ABC technique and evaluated as follows: Cytoplasmic staining for Das-1 in goblet cells was graded as absent (<1% of cells positive) or present (>1% of cells positive). For CG-3, the intensity (absent, weak, strong) and location (i.e. surface epithelium +/- crypt epithelium) of staining in absorptive cells was recorded.

**Results:** Overall, Das-1 goblet cell staining was present in only 1/10 (10%) UC case, in contrast to 7/10 (70%) CD cases (p=0.005) and 9/10 (90%) controls (p=0.002). No significant differences in CG-3 staining intensity were noted between the three groups. However, CG-3 positivity in crypt epithelium was present in 5/10 (50%) UC cases compared to 0/10 (0%) CD cases (p=0.01) and 3/10 (30%) normal controls (p>0.05). The combination of positive CG-3 staining in crypt absorptive cells as well as the absence of DAS-1 staining in goblet cells was noted in 5/10 (50%) UC cases, but in none (0%) of the CD cases (p=0.01) or normal controls (p=0.01). There were no associations between Das-1 or CG-3 staining and any other pathologic (e.g. degree of activity) or clinical features.

**Conclusions:** UC is associated with loss of Das-1 reactivity in goblet cells, whereas CD is associated with loss of CG-3 staining in crypt cells. The reasons for these alterations are unclear. However, the pattern of staining of these antibodies may be clinically useful in the distinction between UC and CD in colonic mucosal biopsies.

### 565 Increased CD68 Positive Macrophages and Macrophage Microaggregates Are Prevalent in Crohn's Colitis and Aid in Its Distinction from Ulcerative Colitis

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**Background:** Previous studies have demonstrated that macrophage microaggregates (MM) are present in gastric mucosal biopsies from patients with Crohn's disease (CD). However, their presence in inflammatory diseases of the colon has never been systematically investigated. The aim of this study was to assess the diagnostic utility of CD68 immunostaining and MM in distinguishing CD from ulcerative colitis (UC).

**Design:** 134 routinely processed colonic mucosal biopsies, including 70 specimens obtained from 20 patients with CD [mean age: 47 yrs, M/F ratio: 1/1], 28 specimens from 20 patients with UC (mean age: 31 yrs, M/F: 1/3) and 36 specimens from 19 normal controls (mean age: 59 yrs, M/F: 8/11) were immunohistochemically stained for CD68 using the ABC technique. The total number of CD68 positive cells in 10 consecutive intercrypt regions (ICR) of the lamina propria was recorded in each biopsy specimen. The number of MM, defined as loose collections of at least 5 macrophages identifiable only by CD68 immunopositivity, and not by H&E staining, present in 10 consecutive ICRs was also recorded. The results between the different patient groups were compared.

**Results:** There were no significant differences in the number of subepithelial or basal lamina propria CD68 positive cells between the three patient groups. However, the mean number of CD68 positive macrophages in the mid lamina propria was higher in CD (28±2) than UC (20±2, p=0.04) and normal controls (22±2, p>0.05). MM were present in 23 biopsies from 13 (65%) CD patients, compared to 5 biopsies from 4 (20%) UC patients (p=0.01) and none (0%) of the normal controls (p=0.0001). Furthermore, only CD cases contained >1 MM (7 biopsies from 4 patients, p=0.01). None of the UC or control patients had >1 MM.

**Conclusions:** Small aggregates of CD68 positive macrophages are more frequently present in CD than in UC and multiple MM are found exclusively in CD. Thus, the presence of multiple MM may aid in the distinction of CD from UC.

### 566 DNA Methylation Profiling of Anal Intraepithelial Lesions and Anal Squamous Cell Carcinoma

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**Background:** Anal intraepithelial neoplasia (AIN) is HPV-associated and may progress to invasive squamous cell carcinoma (SCC), which has recently been detected with increasing frequency in immunocompromised patients. Unfortunately, the biology of AIN is poorly understood and screening programs are not optimal. We hypothesize that AIN is associated with abnormal DNA methylation and that detection of these events may be utilized to improve screening programs.

**Design:** We identified 144 patients who underwent anal cytology screening and subsequent anoscopy and biopsy at our institution between 1999 and 2004 and correlated the cytologic and histologic diagnoses. A subset of these patient specimens were selected for DNA methylation analysis, including 184 anal biopsies (normal, n=57; AIN I (LSIL), n=74; AIN II-III (HSIL), n=41; and SCC, n=12) and 37 residual liquid-based anal cytology specimens (normal, n=11; LSIL, n=12; HSIL, n=14). DNA was extracted from each biopsy and cytology specimen and then bisulfite treated in preparation for real-time methylation-specific PCR (MSP) analysis of the following genes: HIC1, RASSF1, RARb, p16, p14, p73, APC, hMLH1, MGMT, DAPK1, and TSLC1.

**Results:** The histologic diagnoses on the biopsies included 19% normal mucosa, 47% AIN I, and 34% AIN II-III. Cytologic diagnoses on these cases included 5% negative, 30% ASC-US, 55% LSIL, and 10% HSIL. Referral of ASC-US, LSIL or HSIL cytology yielded a sensitivity for detection of biopsy-confirmed HSIL of 100%, but a specificity of 35%. Increasing the threshold of referral to HSIL increased the specificity to 85%, but reduced sensitivity to 25%. Real-time MSP analysis of biopsy samples revealed that aberrant DNA methylation was more common in SCC and HSIL than LSIL and normal mucosa. Specifically, methylation of TSLC1 and DAPK1 occurred at a high frequency in SCC (75% and 75% of cases, respectively) and HSIL (59% and 71%) but was absent in LSIL and normal biopsy samples. Methylation profiles of cytologic samples were similar to those found in the biopsy samples.

**Conclusions:** 1) Anal cytology is a highly sensitive for AIN II-III, but lacks specificity. 2) Aberrant DNA methylation is a frequent event in AIN II-III and anal SCC. 3) Methylation of TSLC1 and DAPK1 is unique to AIN II-III and SCC, and may serve as a useful molecular biomarker. 4) Aberrant DNA methylation can be detected in anal cytology specimens and the methylation profiles resemble those found in biopsies.

### 567 Absent or Low Expression of CK20 and CDX2 Associated with Microsatellite Instability in Poorly Differentiated Colorectal Carcinomas (PDCC)

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**Background:** The expression pattern of cytokeratin 7 (CK7) and cytokeratin 20 (CK20) has been routinely used in discriminating colorectal carcinomas (CK7-/CK20+) from other unknown primaries. Recently, CDX2 was identified as a sensitive and relatively specific marker for primary and metastatic colorectal carcinomas. Reduced expression of CK20 has been demonstrated in colorectal cancer associated with microsatellite instability (MSI). Moreover, MSI and loss of CDX2 expression have recently linked to minimally differentiated colorectal carcinomas. In this study we explore the relationship of CK20, CDX2, and MSI markers in PDCC.

**Design:** Nineteen cases of PDCC (defined as > 80% of the tumor with solid growth pattern with/without dense lymphocytic infiltration) were included in this study. This set of cases represented 1.5% of a large study group of colon cancers. Immunohistochemical stains were performed with monoclonal antibodies to AE1/3, CK7, CK20, CDX2, MSH2 and MLH1. The staining intensity is graded into weak, moderate and strong. The distribution is recorded into negative (<5%), 1+(6-25%), 2+(26-50%), and 3+ (>50%).

**Results:** Of 19 cases, all were strongly positive for AE1/3 and MSH2, and 18 were negative for CK7. Lack of expression of CK20 and CDX2 was present in 53% (n=10) and 58% (n=11) cases respectively. Additionally, low (1+) expression of CK20 and CDX2 was seen in 26% (n=5) and 21%% (n=4) cases respectively. Absence of MLH1 nuclear staining was noted in 68% (n=13) cases, all of which showed absent or low expression of CK20 and CDX2. Notably there were 4 cases that strongly positive for CK20 and also positive for CDX2 and MLH1.

**Conclusions:** This study shows that CK20 and CDX2 have little diagnostic value in differentiating PDCC from other unknown primaries. In addition, the high correlation of expression of CK20, CDX2 and MLH1 suggests a possible role of CDX2 and MLH1 involving the pathway of CK20 gene expression.

### 568 Occult *Helicobacter pylori* Infection Detected by PCR in Biopsies with Chronic Gastritis

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**Background:** *H. pylori* is the main cause of chronic gastritis and peptic ulcer disease and may lead to gastric carcinoma and lymphoma. *H. pylori* gastritis shows a characteristic pattern of inflammation, but only the detection of bacteria proves the disease. Histology is considered a sensitive method, but little is known about the presence of *H. pylori* in inflammed gastric tissue without histological proof of *H. pylori*. We developed two PCR methods to detect *H. pylori* in gastric biopsies and correlated the inflammatory changes with the presence of bacterial DNA.

**Design:** Archived gastric biopsies with at least minor inflammatory changes were included in the study. Biopsies were analyzed for the presence of *H. pylori* DNA by nested and quantitative PCR using primers of the SSA and urease C gene sequence. Biopsies were graded according to the revised Sydney classification.

**Results:** *H. pylori* was detected in 69 (54,8%) of 126 gastric biopsies; 54 (42,9%) by histology and/or PCR and 15 (11,9%) exclusively by PCR. *H. pylori* was found by nested PCR in all samples positive by histology but quantitative PCR failed to detect 10 (18,5%) of 54 biopsies positive by histology. The inflammatory score was significantly higher in biopsies positive only by PCR than in *H. pylori* negative biopsies (mean of neutrophils score 1.60 versus 0.90, p<0.006; mean of mononuclear cells score 2.27 versus 1.67, p<0.006) whereas the results were similar to biopsies positive for *H. pylori* by histology (mean of neutrophils score 1.60 versus 1.56, n.s.; mean of mononuclear cells score 2.27 versus 2.20, n.s.). Quantitative analysis finally showed weak correlation between inflammatory score and the amount of *H. pylori* assesed by histology, but no correlation was found between the inflammatory score and the amount of *H. pylori* DNA detected by quantitative PCR.

**Conclusions:** Our data show that PCR can detect *H. pylori* DNA in a considerable proportion of gastric biopsies with inflammatory changes but negative for *H. pylori* by histology. These biopsies, however, show a degree of inflammation similar to biopsies positive for *H. pylori* by histology, indicating the clinical relevance of *H. pylori* detection by PCR. The sensitivity of our detection systems seems to be depending on the gene region analyzed. However, quantitative analysis provides no additional information.

# Genitourinary

### 569 Non-Hodgkins Lymphoma (NHL) of the Prostate: Emphasis on Useful Diagnostic Features

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**Background:** Hematological malignancies of the prostate are rare with a reported incidence of <1%. The majority of NHL of the prostate were reported using outdated nomenclature prior to the use of immunohistochemistry. We reviewed a series of primary and secondary NHL of the prostate with the aid of immunohistochemistry and used the WHO classification of NHL.

**Design:** The Nebraska Lymphoma Study Group database was searched (1984-2004) for cases of NHL involving the prostate. Cases were classified as primary (no extra-prostatic site identified within one month of diagnosis) or secondary. Clinical and histological material was reviewed to determine clinical presentation, type of specimen, zone of origin, presence of prostatic carcinoma (PCa) and histologic features of the NHL.

**Results:** Ten TURP specimens, 5 needle biopsies and 1 radical prostatectomy were retrieved for review. Clinical presentation was urinary retention and obstruction 7 cases, abnormal DRE 2 and weight loss/hematuria in 1 case each. In 5 cases clinical information was not available. In all cases of primary NHL (12/16) workup was directly related to symptoms of hyperplasia or elevated serum PSA level with only 2 cases having PCa. Zone of NHL orgin was predominantly transitional (10/15). In all cases an immunohistochemistry panel of PSA, PSAP, CD20, CD3, CD5, CD10 and cyclin D1 allowed diagnosis and classification.Types and features of the NHL are presented below:

| WHO Type (n=16) | Pattern of Spread | Necrosis | Intraepithelial Lesions |
|---|---|---|---|
| Diffuse large B-cell (5) | Infiltrative 60% | Present 60% | Present 25% |
| MALT (4) | Nodular 75% | Absent | Present 25% |
| Small lymphocytic (3) | Infiltrative 66% | Absent | Present 66% |
| Intravascular large B-cell (1) | Angiotropic | Absent | Absent |
| Follicular (1) | Nodular | Absent | Present |
| Low grade B cell (1) | Infiltrative | Absent | Absent |
| Mantle cell (1) | Nodular | Absent | Present |

**Conclusions:** NHL of the prostate frequently presents with symptoms of urinary retention (44%) and less often with an elevated serum PSA. In this series, primary NHL of the prostate was an incidental finding in 80% of the cases. Histologically diffuse large B cell was the most frequent (38%) characterized by a infiltrative growth pattern and necrosis (60%). Dense nodular lymphocytic infiltrates were characteristic of MALT and follicular lymphomas (80%). Small lymphocytic lymphoma was characterized by intraepithelial lesions (66%). The presence of nodular, infiltrative and cord like infiltrates and tumor necrosis should raise the possibility of NHL of the prostate and appropriate immunohistochemical markers should be performed.

### 570 Primary Testicular Lymphoma: Retrospective Clinicopathological Study of 34 Cases

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**Background:** Primary testicular lymphoma (PTL) is rare. Before the era of HIV infections it was estimated to comprise 5% of all testicular tumors, with tendency to occur in the elderly. However, more recent reports seem to indicate a higher incidence and a broader age spectrum. PTL are usually aggressive diseases, mostly of B cell immunophenotype. In this study, retrospective clinicopathological review of 34 cases was carried out with emphasis on clinical presentation, immunophenotypic features, and clinical behavior when available.

**Design:** Pathology reports and available material on primary testicular lymphomas seen in 3 medical centers were reviewed. Immunohistochemical stains were performed where these data were lacking and there were available paraffin blocks or unstained paraffin sections. Clinical, pathological and behavioral features were analyzed.