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[654] Expression of HER2 and GRB7 in Upper Gastrointestinal Tract Carcinomas

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- **Background:** Overexpression of HER2 is identified in a subset of gastric and gastroesophageal junction carcinomas, leading to use of trastuzumab based therapy. GRB7, a signaling molecule implicated in tumorigenesis and trastuzumab resistance, is located on the 17q12 amplicon near HER2 and is frequently co-amplified with HER2. The aim of this study was to evaluate immunohistochemical expression of HER2 and GRB7 in upper gastrointestinal tract carcinomas and to correlate these with pathologic stage.
- **Design:** 60 cases of gastroesophageal junction and 34 cases of gastric carcinoma between 1991 and 2006 were selected and immunostained for HER2 and GRB7. HER2 IHC scoring was per published FDA guidelines. 3+ score was considered positive, 0 and 1+ negative and 2+ equivocal. HER2 2+ cases and one GRB7 + HER2 1+ case were submitted for FISH for HER2 amplification. For GRB7 percentage of positively staining cells and intensity of staining were noted. Less than 10% cells with weak staining was considered negative. Results of HER2 and GRB7 were correlated with pathologic stage.
- **Results:** Of the 60 GE junction carcinomas 6 cases (10.0%) were HER2 3+ positive and 7 (11.7%) were HER2 2+ equivocal. GRB7 was positive in 9 cases, distributed as follows: 6 HER2 3+, 2 HER2 2+, 1 HER2 1+. Of the 34 gastric carcinomas 2 cases (6.5%) were HER2 3+ positive and 1 case was HER2 2+ equivocal (2.9%). GRB7 was positive in one case which was also HER2 3+. Overall, GRB7 expression was only seen in conjunction with non-zero HER2 expression, typically at high level (3+, 70%). Of the remaining GRB7+ cases, FISH confirmed amplification in 2 (1 HER2 2+ and 1 HER2 1+, HER2/CEP17 3.79 and 9.36) and showed chromosome 17 polysomy in 1 (HER2 2+ CEP17 3.63). These results indicate that GRB7 is highly specific (90%) for HER2 amplification, with polysomy a possible cause of false positivity.

  Overall, lymph node positivity was 6/7 (86%) in GRB7 positive cases versus only 37/74 (54%) in GRB7 negative cases, though not statistically significant (p=0.11), possibly due to the relative rarity of GRB7 expression.
- **Conclusions:** GRB7 expression is detected in a subset of HER2-amplified cases of upper gastro-intestinal carcinoma, and is rare in unamplified cases. Used in a panel, GRB7 could be used to select cases for FISH when HER2 IHC is 2+ or 1+. Further, this study could be useful for identifying patients who might benefit from potential targeted anti-GRB7 therapy.

  Category: Gastrointestinal

[667] Gastric Biopsies Are Appropriate for Assessment of HER2: A Correlation Study with Resection Specimens

Andrea Grin, Eugene T Hsieh, Christine Brezden-Masley, Catherine J Streutker. St. Michael's Hospital, Toronto, Canada; Sunnybrook Health Sciences Centre, Toronto, Canada; University of Toronto, Toronto, Canada

- **Background:** The survival benefit of trastuzumab in advanced HER2 positive gastric cancer has been demonstrated in a recent large prospective randomized multicentre trial (ToGA trial). Accurate assessment of HER2 status is essential to select patients who will benefit from this therapy. Biopsies are often tested, particularly in a neoadjuvant setting or when patients are inoperable, but little is known on the correlation of HER2 status in biopsy versus resection specimens. While biopsies may have more optimal tissue fixation than resections, gastric cancer has been shown to display significant tumor heterogeneity, which could lead to false negatives in small biopsy specimens. In this study, we sought to examine the diagnostic accuracy of HER2 testing in biopsy specimens.
- **Design:** Forty patients with gastric carcinoma where both initial biopsy and subsequent resection specimen were available were evaluated for the expression of HER2 by immunohistochemistry (4B5, Ventana) by the criteria used in the ToGA trail. FISH (Abbott Molecular) was performed on cases equivocal (2+) by immunohistochemistry and on discrepant cases.
- **Results:** The average number of biopsy fragments was 5.9 with an average of 3.2 fragments positive for carcinoma. Most tumors were intestinal (55%), 15% were diffuse and 30% mixed by the Lauren classification. Overall, 4 of 40 (10%) tumors were HER2 positive, defined as either 3+ by IHC or FISH HER2:CEP17 ratio ≥2.0. Seventy percent (28/40) of patient-paired biopsy and resection specimens showed concordant IHC scores. When 0+ and 1+ scores were grouped and defined as negative, 2+ as equivocal and 3+ as positive, concordance increased to 85% (34/40). Incorporating FISH results in equivocal cases further improved concordance to 95% (38/40). Two cases showed major discrepancies (positive vs. negative overall result), one of which would have resulted in a patient being denied trastuzumab if HER2 was evaluated on the biopsy alone.
- **Conclusions:** Despite tumor heterogeneity in gastric cancer, biopsy specimens are appropriate for evaluation of HER2 when IHC is used along with FISH following the criteria used in the ToGA trial. In our study, there was excellent correlation for HER2 status between biopsy and resection specimens.

  Category: Gastrointestinal

[668] Brightfield Double *In Situ* Hybridization Is Comparable to Fluorescence *In Situ* Hybridization for Determination of HER2 Amplification in Primary Gastric Adenocarcinoma

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- **Background:** HER2 is increasingly being assessed in gastric cancer to identify patients that may benefit from trastuzumab therapy; patients with 3+ HER2 staining on immunohistochemistry (IHC) or amplification by *in situ* hybridization show a significant survival benefit. Fluorescence *in situ* hybridization (FISH) is traditionally used as the gold standard to evaluate HER2 amplification. This method is labour intensive, requires a skilled technologist, and specialized fluorescent microscopy. An alternative is the newly developed automated Brightfield Double *in situ* Hybridization (BDISH) technique and uses differentially labeled Chromosome 17 and HER2 probes that can be read by light microscopy. The aim of this study was to assess the utility of BDISH in gastric cancer and to compare the results to those obtained by IHC and FISH.
- **Design:** Cases were identified from a gastric adenocarcinoma database. Formalin-fixed, paraffin-embedded primary gastric adenocarcinoma specimens were analyzed by IHC (4B5, Ventana), FISH (Abbott Molecular) and BDISH (Ventana). The correlation between methods was calculated.
- **Results:** Results for 48 patients (75% intestinal, 15% diffuse, 10% mixed) were available for analysis. There was a 98% (47/48) concordance rate between BDISH and FISH results (see Table 1). The one discrepant case was scored as 2+ on IHC, was non-amplified by FISH (1.4), but showed amplification by BDISH (2.17). This case displayed tumor heterogeneity, resulting in variable CEP17:HER2 ratios by BDISH depending on the area counted. 71% (5/7) of 3+ cases by IHC were amplified by both BDISH and FISH. All amplified cases were of intestinal type. None of the 0+ or 1+ cases showed amplification by FISH or BDISH (0/31).

IHC-FISH-BDISH correlation					
IHC score	FISH amplified	FISH non-amplified	BDISH amplified	BDISH non-amplified	FISH BDISH concordance (%)
0 (n=20)	0	20	0	20	100
1+ (n=11)	0	11	0	11	100
2+ (n=10)	1	9	2	8	90
3+ (n=7)	5	2	5	2	100
Total	6	42	7	41	98

- **Conclusions:** There is excellent correlation between BDISH and FISH for assessment of HER2 amplification in our cohort. BDISH is rapid, easy to interpret, and offers the added benefit of maintaining cell morphology, enabling assessment of variable amplification areas in tumors displaying heterogeneity. BDISH is a viable alternative to FISH and may identify amplified cases that could be missed by FISH due to heterogeneity in gastric adenocarcinomas.

Category: Gastrointestinal

[674] HER2/Neu Testing in 207 Gastric and Gastroesophageal Junction Adenocarcinomas: Immunohistochemistry and Silver In Situ Hybridization (SISH) Provide Effective Brightfield Methods for Clinical HER2 Testing

Eugene Hsieh, Pauline Henry, Kevin Kwok, Wedad Hanna. Sunnybrook Health Sciences Centre, Toronto, Canada

- **Background:** Advanced gastric and gastroesophageal (GEJ) adenocarcinomas have been shown to overexpress the HER2 receptor in a subset of cases, approximately 10-22%. ToGA, a phase III prospective randomized and multicentre trial, has demonstrated increased overall survival with anti-HER2 therapy, in patients with gastric/GEJ tumours overexpressing the HER2 receptor. Since then, status of the HER2 receptor has become an important biomarker for clinical management of these patients.
- **Design:** Paraffin blocks from 187 primary and secondary gastric/GEJ adenocarcinoma biopsy specimens from 1999-2011 were retrieved from the archives of Sunnybrook Health Sciences Centre (SHSC), and were tested for HER2 expression using both immunohistochemistry (IHC) (Ventana PATHWAY anti-HER-2/neu 4B5), and silver in situ hybridization (SISH) (Ventana INFORM HER2 and chromosome 17 DNA probe kits). In addition, 20 consecutive outside referral biopsy specimens from 2011 were also tested, as above. IHC was scored from 0-3+ using published gastric cancer IHC interpretation criteria, with 0-1+ negative, 2+ equivocal, and 3+ positive. SISH positive (SISH+) was defined as HER2/chr.17 ratio ≥ 2.0.

- **Results:** 28/187 (15.0%) SHSC cases were SISH+. Of these, 19/28 (67.9%) were IHC 3+ (positive) and 7/28 (25%) were IHC 2+ (equivocal). 2/28 (7.1%) were IHC 1+ (negative). No SISH+ cases were IHC 0. ($p < 0.0001$). For the outside referral cases, 3/20 (15.0%) had SISH overexpression, with all three cases IHC 3+. Overall 29/31 SISH+ cases demonstrated IHC 2+ or 3+ expression, with only 2/31 SISH+ cases demonstrating IHC 1+ expression. All SISH+ cases were of intestinal or mixed type. No pure diffuse type cancers had IHC 3+ expression, and all were uniformly SISH negative.
- **Conclusions:** Since the approval of anti-HER2 therapy for gastric and GEJ cancer, HER2 testing has become necessary for patient management decisions. Traditional testing protocols have used IHC with secondary fluorescence based in situ hybridization testing when necessary. This study demonstrates excellent concordance of SISH+ and SISH- status with IHC 3+ and IHC 0 respectively, and very good concordance of SISH- status with IHC 1+. Thus, in the clinical setting, IHC can be used primarily, with reflex SISH testing of equivocal IHC 2+ cases and possibly IHC 1+ cases. This combination of IHC plus SISH provides an excellent brightfield-only alternative, allowing adoption in a greater number of pathology laboratories than is possible with fluorescence based techniques.

  Category: Gastrointestinal

[657] Loss of SDHB Expression Is Limited to a Distinctive Subset of Gastric Wild-Type Gastrointestinal Stromal Tumors: A Comprehensive Genotype-Phenotype Correlation Study

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- **Background:** Gastrointestinal stromal tumors (GISTs) typically harbor activating mutations in *KIT* or *PDGFRA*; however, 15% of GISTs in adults and >90% in children lack such mutations ["wild-type" (WT) GISTs]. Pediatric WT GISTs, GISTs in patients with Carney triad and Carney-Stratakis syndrome, and some WT GISTs in adults show similar, distinctive clinical and pathologic features. These "pediatric-type" ("type 2") GISTs occur in the stomach, may be multifocal, show multinodular/plexiform architecture and epithelioid morphology, often spread to lymph nodes, are imatinib resistant, and tend to pursue an indolent clinical course even with metastatic disease. Recent studies have suggested that this distinctive group of tumors can be identified by loss of succinate dehydrogenase subunit B (SDHB) protein expression by immunohistochemistry (IHC). The aim of this study was to validate the predictive value of SDHB IHC for this subset of WT GISTs in a large genotyped cohort.
- **Design:** SDHB protein expression was examined by IHC in whole tissue sections from 261 GISTs with known *KIT* and *PDGFRA* genotypes: 179 tumors with KIT mutations (154 in exon 11, 17 in exon 9, 4 in exon 13, and 4 in exon 17), 31 tumors with *PDGFRA* mutations (24 in exon 18, 4 in exon 12, and 3 in exon 14), and 51 WT tumors. IHC was performed following antigen retrieval using a mouse anti-SDHB monoclonal antibody (1:100; 21A11AE7; Abcam). Cytoplasmic staining was scored as "intact" or "deficient". Histologic features were recorded without knowledge of genotype or SDHB status.
- **Results:** SDHB expression was deficient in 20 (39%) WT GISTs. All other tumors showed intact expression of SDHB, including 100% of *KIT* and *PDGFRA*-mutant GISTs and 31 (61%) WT GISTs. All SDHB-deficient GISTs with known primary site (N=19) arose in the stomach and had a multinodular/plexiform growth pattern and epithelioid or mixed morphology. Of the WT GISTs with intact SDHB expression, 10 arose in the small intestine, 7 in the stomach, 5 in the colon, and 1 in the esophagus; primary site was unknown in 8 cases. None showed a multinodular architecture, and only 4 (13%) had epithelioid morphology.
- **Conclusions:** SDHB-deficient GISTs are WT gastric tumors with distinctive features that can be recognized histologically. IHC for SDHB can be used to confirm the diagnosis of this class of tumors, which has prognostic, therapeutic, and syndromic implications. SDHB expression is retained in all GISTs with *KIT* and *PDGFRA* mutations.

  Category: Gastrointestinal

PATOLOGÍA TUMORAL DE INTESTINO GRUESO

[721] Silencing of P16 ^{Ink4a} in Colorectal Cancer Is Associated with *BRAF* Mutation and Independent of Microsatellite Instability

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- **Background:** Sporadic colorectal cancer (CRC) with high-level microsatellite instability (MSI-H) is strongly associated with BRAF mutation and the CpG island methylation (CIMP) phenotype. However, the causal relationship between *BRAF* mutation and CIMP is not well understood. p16, a cell cycle inhibitor, is a specific marker of CIMP phenotype. Silencing of p16 is often caused by promoter methylation of CDKN2A gene in CRC. Recent study suggested that p16 is a marker of oncogene-induced senescence and is expressed in the precursor lesions of the serrated neoplasia pathway. We hypothesize that p16 silencing in CRC correlates with *BRAF* mutation independently of MSI-H status.
- **Design:** Consecutive colectomy specimens for CRC were collected from 98 patients. *KRAS* mutations (codons 12/13), *BRAF* mutation (V600E), MSI status (NCI panel of 5 markers) and p16 immunohistochemical expression were evaluated on representative paraffin blocks. Additional staining for p16 was also done in normal colon, conventional adenomatous polyps (APs) and sessile serrated adenomas (SSAs). A case was considered positive for p16 if 5% or more cancer cells had nuclear and cytoplasmic staining.

- **Results:** Based on the mutation status of *BRAF* and *KRAS*, 98 CRC cases were classified into 3 groups [Table 1]. Group 1(n=16, mean age 76) harbored *BRAF* mutation. Lack of p16 expression was seen in all but one (94%, n=15) cases, of which 10 were MSI-H and 5 were MSS (microsatellite stable). Group 2 (n=33, mean age 68) exhibited KRAS mutations. Five cases (15%) did not express p16. These 5 cases were either MSI-H (n=1) or MSS (n=4). Group 3 (n=49, mean age 64) contained wild types *BRAF* and *KRAS* genes. Ten cases (20%) lacked the expression of p16, of which nine were MSS and one was MSI-H.

  Normal colonic mucosa (n=5) was negative for p16. Patchy p16 positivity was present in the lower crypts of SSA (n=6) and various areas in AP (n=8).

Table 1 Expression of p16 in CRC

	Group 1	Group 2	Group 3
Mean age (range)	76 (58-89)	68 (29-92)	64 (22-93)
BRAF	Mutated	Wild type	Wild type
KRAS	Wild type	Mutated	Wild type
Total case no.	16	33	49
p16 negative case no	15 (94%) ¶§	5 (15%) ¶	10 (20%)§

¶§ p value<0.001 (chi square test)

- **Conclusions:** Silencing of p16, a specific CIMP marker, in CRCs is highly associated with *BRAF* mutation independently of microsatellite instability. These findings suggest that CIMP phenotype is a property of *BRAF* mutation rather than MSI status. Future studies of p16 and other markers related to *BRAF* or *KRAS* mutations in CRC may increase our understanding on the biological behaviors and cell cycle controls of CRC.

  Category: Gastrointestinal

[664] Assessment of MLH1 Promoter Methylation and BRAF Gene Mutation in Colorectal Carcinomas with Microsatellite Instability

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- **Background:** Recent studies indicate that analysis of MLH1 promoter methylation and especially evaluation of BRAF gene mutational status can be employed to differentiate hereditary from sporadic MSI-H MLH1-negative colorectal carcinomas. In particular BRAF was demontrated to be frequently mutated in MSI-H sporadic but not in hereditary carcinomas.
- **Design:** The study was conducted on a consecutive series of 2162 colorectal adenocarcinomas surgically resected from January 2004 to June 2010. Mismatch repair (MMR) status has been prospectively evaluated by immunohistochemical analysis of MMR protein expression (MLH1, MSH2, MSH6 and, in selected cases, PMS2) and microsatellite instability (MSI) analysis, using a fluorescent PCR method and the Bethesda panel markers (BAT25, BAT26, D2S123, D5S346, D17S250) plus BAT40. Tumors were classified as MSI-H, MSI-L and MSS according to the guidelines of the International Workshop of Bethesda. In MMR-deficient (MMR-D) tumors, analysis of MLH1 promoter methylation (C- region) was assessed by methylation specific PCR and evaluation of V600E BRAF mutation was investigated by direct DNA sequencing.
- **Results:** 316 (14.6%) carcinomas were classified as MMR-D (loss of MMR protein expression and/or MSI-H). Most MMR-D tumors showed loss of MLH1 expression (256, 81%). MLH1 methylation was detected in 196/219 (89%) MLH1-negative carcinomas and in 2/50 (4%) MMR-D MLH1-positive carcinomas. V600E BRAF mutations were observed in 108/158 (68%) MLH1-negative and in only 1/42 (2%) MLH1-positive MMR-D cancers. BRAF mutations were identified only in tumors showing MLH1 promoter methylation (107/142, 75%). All the MLH1-negative carcinomas without MLH1 methylation examined (15 cases) did not demonstrate BRAF mutation. Both MLH1 promoter methylation and BRAF mutation were more frequently observed in older patients.
- **Conclusions:** Our results confirm that MLH1 promoter methylation and BRAF mutation occur in a large fraction of MMR-deficient MLH1-negative colorectal carcinomas and are closely associated. Furthermore our data indicate that assessment of MLH1 promoter methylation and especially of BRAF mutation might be used in the selection of colorectal cancer patients with presumptive Lynch syndrome.

  Category: Gastrointestinal

[722] Sporadic Colonic Adenocarcinomas with a High Degree of Microsatellite Instability (MSI-H) Do Not Show Evidence of Wnt Signaling Abnormalities

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- **Background:** Most sporadic colonic adenocarcinomas arise from adenomas *via* dysregulation of the Wnt signaling pathway, as evidenced by nuclear B-catenin immunostaining, and are microsatellite stable (MSS). Sessile serrated polyps are not dysplastic, but do show *BRAF* mutations and DNA methylation, and have been implicated in the serrated neoplastic pathway. Onset of MSI-H in serrated polyps coincides with progressive dysplasia and leads to sporadic cancers with *BRAF* mutations, MSI-H, and widespread promoter hypermethylation. Wnt signaling and MSI-H are generally considered to be mutually exclusive cancer progression models. However, several studies have described abnormal B-catenin staining in serrated polyps, suggesting a role for Wnt signaling in development of sporadic MSI-H cancers. We evaluated B-catenin staining in sporadic colon cancers to determine whether Wnt activation promotes carcinogenesis in MSI-H tumors.
- **Design:** 43 colon cancer resection specimens were evaluated. All patients were >60 years old with tumors proximal to the splenic flexure. Immunostains for MLH1, PMS2, MSH2, MSH6, and B-catenin were performed. Aberrant B-catenin staining was defined by moderate-to-strong nuclear, and diminished membranous, staining in ≥30% of the tumor cells. Tumor DNA was extracted and assessed by PCR for MSI using 5 mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24, MONO-27). Pyrosequencing was carried out to assess for *BRAF* codon V600E mutations.
- **Results:** 23 tumors showed complete loss of MLH1 and PMS2 staining with MSI-H, including 16 (70%) with *BRAF* mutations (M/F=6/17, mean age: 72 years). Only 1 (4%) MSI-H tumor displayed aberrant B-catenin staining and it was *BRAF* wild-type. Twenty cancers had preserved staining for DNA repair proteins, MSS, and wild-type *BRAF* (M/F: 11/9, mean age: 71 years). Of these, 75% showed abnormal B-catenin staining, which was significantly more than MSI-H tumors ($p<0.0001$).
- **Conclusions:** MSS colonic carcinomas are usually *BRAF* wild-type and show nuclear localization of B-catenin with diminished cell membrane staining, reflecting aberrant Wnt signaling. Abnormal B-catenin staining is rare in sporadic MSI-H cancers and, in fact, we found no abnormal B-catenin expression among MSI-H cancers with *BRAF* mutations. Despite reports of nuclear B-catenin accumulation in serrated polyps, we conclude that Wnt signaling alterations are unlikely to be biologically important in sporadic MSI-H tumors.

Category: Gastrointestinal

[758] Clinicopathologic and Molecular Characterization of PIK3CA Mutations in Colorectal Neoplasms

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- **Background:** PIK3CA mutations have been described in 10-20% of colorectal carcinomas. Only a small number of studies have examined their significance, often with conflicting results.
- **Design:** We analyzed frozen tissue from 613 colorectal neoplasms including 34 adenomas for hotspots mutations in PIK3CA (exons 4, 7, 9 and 20), KRAS, and BRAF using a MALDI-TOF mass-spectrometry based genotyping assay. TP53 mutations were also detected using Sanger sequencing. Microsatellite instability testing was performed in 352 cases and clinicopathologic data was collected in all cases. Mutant allele frequency (MAF) was determined in 2 cases using deep sequencing.
- **Results:** PIK3CA mutations were detected in 69 (11.3%) cases including 5 (14.7%) adenomas and the majority of mutations were in exon 9 (n=44, 63.8%). Right-sided tumors were more frequently mutated compared to left-sided and rectal tumors (16.7% vs 9.6% and 9.7%, $p=0.03$), but there was no association between PIK3CA mutations and sex, age or stage at presentation. PIK3CA mutations were more common in microsatellite unstable (MSI-H) tumors (29% vs. 9.7%, $p<0.001$), and were observed both in sporadic and hereditary non-polyposis colorectal cancer-associated cases. Compared to PIK3CA wild-type tumors, PIK3CA-mutated tumors were more likely to harbor KRAS mutations (16.1% vs. 7.7%, $p=0.002$). There was no significant association between PIK3CA and TP53 or BRAF mutations, although none of the tumors with PIK3CA exon 20 mutations harbored a BRAF mutation. In 2 KRAS/PIK3CA mutant cases tested the PIK3CA MAF was lower compared to the KRAS MAF, suggesting the PIK3CA mutation occurred later than the KRAS mutation. There was no significant difference in disease specific survival between PIK3CA mutant and wild type tumors even after MSI-H tumors were excluded from analysis.
- **Conclusions:** PIK3CA mutations are present in adenomas, suggesting that they occur relatively early in colorectal carcinogenesis. They show a strong association with KRAS mutations and are more frequent in right-sided and MSI-H tumors. Contrary to a prior study, we did not observe an association with adverse prognosis.

Category: Gastrointestinal

[754] Reproducibility of the Diagnosis of Malignant Colorectal Polyps

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- **Background:** Due both to introduction of screening of colorectal cancer and improvement of endoscopically techniques, the resection of malignant polyps (Tis and T1) is increasing. On a limited specimen, the pathologist has the critical task of determining whether there is a significant risk of recurrence or metastasis which may result in complementary surgical resection of the colorectum. To date, however, the level of agreement among pathologists in diagnosing malignant polyps has not been well established.
- **Design:** Using a web-based virtual microscopy, we examined the observer variation in the diagnosis of 100 malignant colorectal polyps (Tis and T1) endoscopically resected by 13 gastrointestinal pathologists. Different pathological parameters were assessed as tumor differentiation, angiolymphatic vessel invasion, tumor budding, Haggitt's or Kudo's levels, measurement of submucosal invasion and base resection margin status. Results were analyzed by kappa (k) statistics and for percentage agreement.
- **Results:** k analysis indicated that the strength of agreement was substantial for resection margin (k=0.61), moderate for pTis/pT1 classification (k=0.54), tumor budding (k=0.44), and slight for tumoral differentiation (k=0.14). Interobserver agreement was fair and moderate for Haggitt's and Kudo's classifications respectively. A better agreement was observed when measurement of the width or depth of submucosal invasion was performed (k=0.58 and k=0.54). According to the presence or not of pejorative criteria, there was a moderate agreement among pathologists on patient management strategies (k=0.54). A better agreement was observed among pathologists working in most active endoscopic centers and for specimens well orientated.
- **Conclusions:** Gastrointestinal pathologists demonstrate moderate agreement for differentiate intramucosal carcinomas (pTis) from invasive adenocarcinomas (pT1) in endoscopically colonic resected specimens. The high interobserver variability concerning the tumoral differentiation parameter is clinically irrelevant. Measurement of the extent of invasion in submucosa appears more reproducible than the Haggitt's and Kudo's classifications.

Category: Gastrointestinal

[732] MACC1, a Potential Diagnostic Marker for Early Stage Colorectal Cancer

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- **Background:** Metastasis-Associated in Colon Cancer 1 (MACC1), a newly identified gene, may act as a key regulator of the HGF-MET pathway, and is associated with both malignant and metastasis of colorectal carcinoma (CRC). To explore potential markers for early detecting malignancy and predicting metastasis of CRC, we evaluate the expression of MACC1 and MET in adenoma, adenoma with high grade dysplasia, malignant polyps and adenocarcinoma with metastasis.
- **Design:** A total of 48 cases were analyzed, including 13 tubular adenomas, 11 adenomas with high grade dysplasia, 19 malignant polyps (15 intramucosal, 4 invasive adenocarcinoma), and 5 adenocarcinomas with distant metastasis. MACC1 and MET expression were investigated by immunohistochemical method. Cytoplasmic staining for each protein was semiquantified as negative (0), positive with low level expression (1) or positive with high level expression (2). The expression of MACC1 and MET in different groups was evaluated and statistically analyzed.
- **Results:** Positive MACC1 staining was seen in 15% of adenoma, 63% of adenoma with high grade dysplasia, 89% of malignant polyps (13 intramucosal and 4 T1 invasive carcinoma) and 100% of invasive carcinoma with metastasis ($P<0.01$). Furthermore, high level MACC1 expression was observed in all (5/5) of carcinoma with metastasis and 16% (3/19) of malignant polyps, but not in adenoma or adenoma with high grade dysplasia ($P<0.01$). Although no significant different expression of MET was found among experimental groups, the correlation between MACC1 and MET expression was seen in carcinoma with metastasis. 80% of metastatic carcinoma showed high expression of both MACC1 and MET. On 10 to 53 months follow up, 7 of 19 of patients with malignant polyps had subsequent segment resection, none of the 19 patients with malignent polyps develops distant metastasis.
- **Conclusions:** Our study suggests that MACC1 may be an important indicator for malignant transition from adenoma to high grade dysplasia, and invasive adenocarcinoma. The expression of MACC1 may serve as a marker for early diagnosis of colorectal cancer. High expression of both MACC1 and MET in colorectal adenocarcinoma with metastasis confirmed the essential roles of these two proteins in the process of cancer metastasis. Met expression does not have diagnostic value in early colorectal malignancy.

Category: Gastrointestinal

[678] Analysis of LGR5 Immunohistochemical Expression in Gastrointestinal Neuroendocrine Tumors

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- **Background:** LGR5, a recently de-orphanized G-protein couple receptor (GPCR) and Wnt pathway regulator, was shown to be expressed in murine intestinal stem cells. In human small intestine, LGR5 stains rare crypt base cells with neuroendocrine features. The expression of LGR5 in tumors with neuroendocrine differentiation has not been reported yet. We therefore aimed to study the expression of LGR5 in gastrointestinal neuroendocrine tumors.
- **Design:** A tissue microarray of 44 gastrointestinal neuroendocrine tumors and 57 hepatic neuroendocrine tumor metastases were stained for LGR5 by immunohistochemistry (ab12827, Abcam; K5361, Dako). Thirty-four paired cases of primary tumors with their metastases were included. Normal pancreas and small intestine were used as control for staining. The LGR5 expression pattern was scored based on staining intensity and percentage of positive cells. The LGR5 staining was compared with the Ki67 index and the reported chromogranin and synaptophysin markers.
- **Results:** In normal pancreas, LGR5 is strongly expressed in neuroendocrine (islet) cells. 88% of the primary (3/4 gastric, 23/24 intestinal, 13/14 pancreatic, 0/2 appendiceal) and 87% of the metastases stain positive for cytoplasmic LGR5. 67% of the tumors show similar LGR5 expression levels in their metastases. LGR5 is positive in the majority of the cases expressing chromogranin and synaptophysin (34/38). More interestingly, LGR5 was positive in the 7 cases negative for chromogranin and 5 negative for synaptophysin. In our study, twenty-three of the thirty seven cases with a Ki-67 index greater than 2% show weak or absent LGR5 expression. Only fourteen of the sixty two cases with a Ki67 index less than 2% have weak or absent LGR5. Thus, the partial or complete loss of expression correlates with an increased Ki67 index (>2%) (Fisher's exact test, $p<0.05$).
- **Conclusions:** LGR5 is a novel immunohistochemical marker for gastrointestinal neuroendocrine tumors. LGR5 appears to also retain positivity in tumors that lost classical neuroendocrine marker expression. A possible explanation could be that LGR5 is a marker of early neuroendocrine differentiation that maintains its expression after other markers are lost. Interestingly, in our study, the loss of LGR5 expression correlates with an increased Ki67 index, therefore we suggest that it may be an indicator of poorer outcome.

  Additional studies are needed to further confirm marker specificity. Given the widespread use of GPCR-targeting drugs in medicine, LGR5 may represent a novel target for neuroendocrine tumor chemotherapy.

  Category: Gastrointestinal

[630] Immunohistochemical Features of Intestinal and Foveolar Dysplasia in Barrett's Esophagus

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- **Background:** Recently, two major subtypes of dysplasia in Barrett's Esophagus (BE) have been identified, termed "intestinal" (INT) or "gastric", also referred to as "foveolar" (FOV). Some patients show a mixture of both types. Previous data have shown that these two subtypes of dysplasia may have different biological characteristics, arise from different types of BE mucosa, and may have different natural history and risk of malignancy. The aim of this study was to determine the immunohistochemical characteristics of these dysplasia subtypes in BE, with emphasis on the type of differentiation (intestinal vs. gastric) present in both.
- **Design:** Thirty-eight BE-related endoscopic mucosal resections (EMR) were evaluated morphologically for the highest grade (low, high) and type of dysplasia (INT, FOV, mixed) by routine H&E histologic methods. Immunohistochemistry was performed for intestinal markers (CDX2, MUC2, and villin) and gastric markers (MUC5AC, MUC6) and for Ki67 and P53, and all markers were scored for the presence and degree of staining in a semiquantitative fashion (grade 0=negative, 1=focal, 2=multifocal, 3=diffuse staining). Staining was compared between the different types of morphologic dysplasia.
- **Results:** By morphology, 11 (29%) were considered intestinal, 8 (21%) foveolar, and 16 (42%) mixed dysplasia. By immunohistochemistry, morphologically classified INT dysplasia showed significantly higher expression of INT markers, such as MUC2, CDX2, and villin, whereas FOV dysplasia showed significantly more MUC5AC and MUC6 expression. Mixed INT/FOV dysplasia showed an immunophenotypic pattern that matched, roughly, the morphologic areas of INT and FOV dysplasia. No significant differences were observed in P53 or Ki67 proliferative index. Interestingly, despite differences in quantity of the various INT and gastric immunomarkers in INT and FOV dysplasia, respectively, all cases of both types of dysplasia showed at least focal CDX2 staining.

- **Conclusions:** Regardless of the morphologic phenotype of dysplasia, whether INT or FOV, all dysplastic epithelium shows some evidence of intestinalization characterized by at least focal CDX2 staining, similar to the background non-dysplastic metaplastic columnar epithelium as previously reported. In general, INT dysplasia shows more advanced INT immunophenotype, in contrast to FOV dysplasia which shows a more advanced gastric phenotype. Further studies are needed to determine whether dysplasia types with more advanced INT versus gastric differentiation have a different risk of malignancy.

  Category: Gastrointestinal

[747] Biopsies from the GEJ Area Composed of Pure Oxyntic Glands Is Not Necessarily Indicative of the Proximal Stomach

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- **Background:** Barrett's esophagus (BE) has been proposed to develop via conversion of mucosa comprised of oxyntic glands to one comprised of mucous glands prior to goblet cell metaplasia. Based on this theory, some authorities have suggested that biopsies from the GEJ area composed of pure oxyntic glands always represent mucosa from the proximal stomach. The aim of this study was to evaluate the prevalence and significance of mucosa composed of pure oxyntic glands when detected in biopsies of the GEJ region in a large study of GERD patients either with (N=214) or without (N=295) columnar-lined esophagus (CLE).
- **Design:** Biopsies of the GEJ region from 509 GERD patients (M/F ratio: 281/228, mean age: 51 years), all of whom were endoscoped and interviewed prospectively as part of a community clinic-based study of GERD patients in Washington state, were evaluated in a blinded fashion for the presence or absence of pure oxyntic-type mucosa and goblet cells, without knowledge of the clinical characteristics or anatomic location (esophagus or stomach) of the biopsy. Patient endoscopic features, presence or absence of CLE and clinical risk factors for BE, such as gender, race, waist/hip ratio, smoking history, severity of GERD symptoms, and body mass index (BMI) were compared between patients with vs without pure oxyntic-type mucosa and, of the former, with or without goblet cells.
- **Results:** Biopsies composed of pure oxyntic glands occurred in 126/509 (24.8%) patients overall. Pure oxyntic-type mucosa was significantly more common in patients without CLE (30.5%) compared to patients with CLE (18.9%, p=0.003), and no significant difference was observed in short (21.8%) vs long segment (15.8%) type. Goblet cells occurred in 11/126 (8.7%) cases, and no significant differences were observed in CLE (12.5%) vs non-CLE (7%). No measured risk factors for BE or demographic characteristics of the patients were significantly related to the presence of pure oxyntic-type mucosa, or to those cases with or without goblet cells with one exception; males (80%) were more likely to have pure oxyntic-type mucosa vs females (68%, p=0.004).
- **Conclusions:** Pure oxyntic-type mucosa may occur in a substantial proportion of esophageal biopsies in patients with CLE; thus, mucosa with this phenotype does not necessarily represent tissue from the proximal stomach. Contrary to the prevailing theory of BE pathogenesis, in some cases, goblet cells may be derived directly from pure oxyntic-type mucosa, without an intervening stage of mucous gland metaplasia.

  Category: Gastrointestinal

[764] Poor Agreement for Detection of Goblet Cells in Esophageal and GEJ Biopsies

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- **Background:** The American Gastroenterological Association (AGA) criteria for Barrett's esophagus (BE) includes endoscopic recognition of columnar mucosa and histologic confirmation of goblet cells in mucosal biopsies. Goblet cells are often few in number, and may be difficult to differentiate from pseudogoblet cells. This study was performed to determine the interobserver variability for detection of goblet cells, and other cell types, in biopsies from the distal esophagus and GEJ.
- **Design:** Digitally scanned mucosal biopsies (Aperio system) from 34 patients, obtained from either the distal esophagus (N=16, 5 long segment BE, 11 short segment BE) or GEJ (N=18) were evaluated by 7 GI pathologists for a variety of histologic features, such as goblet cells, multilayered epithelium (ME), pseudogoblet cells, and type of glands, and then the pathologists were asked whether they believed the biopsies were diagnostic of BE. Overall, 18 cases with few, or only rare, goblet cells diagnosed by the original signout pathologist, who did not participate further in the interobserver study, were selected for study. The data were analyzed by Kappa statistics.

- **Results:** Overall interobserver agreement for detection of goblet cells was poor (Kappa = 0.35), and agreement was worse for esophageal versus GEJ biopsies. All 7 reviewing pathologists agreed on the presence of goblet cells in only 12 cases. Kappa values for detection of ME and pseudogoblet cells were very poor (0.29 and 0.10, respectively). The highest levels of agreement were obtained for detection of gland type (Kappa = 0.54). However, even regarding gland type, all 7 pathologists agreed on the presence of mucous glands (vs. oxyntic vs. mixed mucous/oxyntic) in only 29% of cases. Agreement regarding whether the observers believed the biopsy fulfilled the histologic criteria for BE was extremely poor (Kappa=0.15).
- **Conclusions:** Based on the results of this study, the current AGA criteria for BE is problematic, since interobserver agreement for detection of goblet cells when the latter are few in number is poor.

  Category: Gastrointestinal

PATOLOGÍA PANCRÁTICO-BILIAR

[768] Increased IgG4+ Cells in Duodenal Biopsies Are Not Specific for Autoimmune Pancreatitis

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- **Background:** Endoscopic ampullary biopsies showing increased IgG4+ plasma cells have been recently reported as an alternative to pancreatic biopsy in diagnosing autoimmune pancreatitis. In some institutions, pathologists are being asked to perform IgG4 immunostaining on duodenal biopsies without clear clinical indications or clear guidelines on how to interpret the significance of finding an increase in IgG4+ cells. The purpose of this study was to assess whether increased IgG4+ cells can be seen in duodenal biopsies outside the context of autoimmune pancreatitis.
- **Design:** Duodenal biopsies from 28 patients undergoing endoscopy for suspected celiac disease (16), abdominal pain (6), recurrent pancreatitis (1), inflammatory bowel disease (IBD) surveillance (3), or post-*Helicobacter pylori* treatment (2) were stained immunohistochemically for IgG4.
- **Results:** All duodenal biopsies that were histologically normal (n=5) or showed increased intraepithelial lymphocytes (IELs) without villous blunting (n=8) were negative for IgG4. Scattered IgG4+ cells (up to 10 per hpf) were found in 5 cases of 10 serologically confirmed celiac disease patients, with higher numbers of IgG4+ cells in biopsies showing significant villous blunting. In addition, there was heavy IgG4 positivity (10 to >30 per hpf) in 5 patients who had normal pancreata on ultrasound; 1 patient with recurrent pancreatitis and ulcerative colitis, 1 patient with primary sclerosing cholangitis (PSC) and ulcerative colitis, and 3 patients with confirmed celiac disease. Of these patients, the duodenal biopsies from the recurrent pancreatitis patient showed gastric heterotopia; her serum IgG4 was normal. In the PSC patient, the biopsies showed villous blunting, increased IELs, ulcer, and foveolar metaplasia. Biopsies of the celiac disease patients also showed severe villous blunting and increased IELs.
- **Conclusions:** The finding of increased IgG4+ cells in duodenal biopsies is not specific for autoimmune pancreatitis. Even with a large number of IgG4+ cells, appropriate clinical findings and radiologic correlation are necessary to make a diagnosis of autoimmune pancreatitis. In our study, scattered IgG4+ cells were found particularly in duodenal biopsies with villous blunting and increased IELs, with 5 cases showing dense IgG4+ infiltrates. The significance of IgG4 positivity in celiac disease and in IBD is unclear. Nevertheless, the sole finding of increased IgG4+ plasma cells in biopsies is not specific for IgG4-related sclerosing disease, as seen in this study and reports of other conditions such as gastritis in pernicious anemia and in rheumatoid arthritis.

  Category: Gastrointestinal

[713] Glypican-3 Expression in Gastrointestinal and Pancreatic Carcinomas

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- **Background:** Glypican-3 (GPC3), a cell membrane bound proteoglycan that can be overexpressed in certain malignancies, has been particularly linked to hepatocellular carcinoma (HCC). GPC3 is currently used as an immunohistochemical (IHC) marker of HCC but its expression in carcinomas of the gastrointestinal (GI) tract and pancreas, a common source of liver metastasis, has not been studied in detail. This study aims to evaluate GPC3 expression in carcinomas of the GI tract and pancreas.
- **Design:** We examined IHC expression of GPC3 in 158 carcinomas including 52 adenocarcinomas (ADCA) of the pancreas and GI tract (8 esophagus, 8 stomach, 2 small bowel, 12 colon), 29 squamous cell carcinomas (SCC) of the GI tract (19 esophagus and 10 anus), 65 neuroendocrine carcinomas (NECA) of the pancreas and GI tract (2 esophagus, 8 stomach, 15 small bowel, 14 colon), and 12 pancreatic acinar cell carcinomas. Two control groups (32 HCC and 16 intrahepatic cholangiocarcinomas) were also stained with GPC3. All tumors were scored for cytoplasmic and membranous staining. A tumor was considered positive for GPC3 if >10% of neoplastic cells showed strong cytoplasmic and membranous immunoreactivity.

- **Results:** 22 of 158 extrahepatic tumors (14%) were positive for GPC3. In the hepatic tumor group, none (0/16) of the cholangiocarcinomas and 24/32 (75%) of HCC were GPC3 positive. In extrahepatic tumors, GPC3 immunoreactivity was most frequent in pancreatic acinar cell carcinomas (58.5%), followed by SCC (27.5%), and ADCA of theGI tract (20%). All pancreatic ADCAs and NECAs were GPC3 negative. GPC3 expression correlated with poor differentiation in HCC but no such correlation was present in extrahepatic tumors. GPC3 positivity was more frequent in upper GI tract ADCA (28%) compared to lower GI tract ADCA (8.5%). In all tumors including HCC, GPC3 expression showed no significant correlation with tumor size. In all positive tumors, GPC3 immunoreactivity was characterized by strong cytoplasmic and membranous staining of 20-100% of the neoplastic cells. Diffuse positivity occupying 100% of tumor cells was only observed in HCC and pancreatic acinar cell carcinoma. SCCs typically demonstrated a predominant peripheral/basal distribution of GPC3 immunoreactivity.
- **Conclusions:** GPC3 immunoreactivity occurs frequently in carcinomas of the GI tract and pancreas and is most often observed in pancreatic acinar cell carcinoma, SCC, and ADCA of the upper GI tract. As these tumors commonly metastasize to the liver, this can lead to a mistaken diagnosis of HCC; GPC3's lack of specificity should be recognized when evaluating tumors involving the liver to avoid potential diagnostic pitfalls.

  Category: Gastrointestinal