Patología Digestiva

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765
TTF-1 Is Expressed in a Subset of Esophageal Adenocarcinomas

AC Layne, TJ Cummings, CD Guy, DM Cardona, RC Bentley, MJ Shealy, X Zhang, SJ McCall. Duke University, Durham, NC.
Background:
Distinguishing primary esophageal adenocarcinoma (EAC) from direct extension of primary pulmonary adenocarcinoma into the esophagus can be difficult in locally advanced tumors.
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Distinguishing primary esophageal adenocarcinoma (EAC) from direct extension of primary pulmonary adenocarcinoma into the esophagus can be difficult in locally advanced tumors. Caudal-type homeobox 2 (CDX2) has 80% sensitivity and excellent specificity for EAC, and thyroid transcription factor-1 (TTF-1) is a sensitive marker for pulmonary adenocarcinoma.
Background:
Distinguishing primary esophageal adenocarcinoma (EAC) from direct extension of primary pulmonary adenocarcinoma into the esophagus can be difficult in locally advanced tumors. Caudal-type homeobox 2 (CDX2) has 80% sensitivity and excellent specificity for EAC, and thyroid transcription factor-1 (TTF-1) is a sensitive marker for pulmonary adenocarcinoma. Three studies, totaling 26 individual cases, have described a complete lack of TTF-1 positive EAC cases. Thus, CDX2 and TTF-1 make an attractive panel to differentiate between these two tumor types.
Desing:
Resection specimens from 24 primary EACs were identified in our database after IRB approval. None of the patients had a known history of pulmonary adenocarcinoma. IHQ: TTF-1, CDX2, and CK7 in all cases (CK20 and villin staining utilized in difficult or ambiguous cases).
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IHQ: TTF-1, CDX2, and CK7 in all cases (CK20 and villin staining utilized in difficult or ambiguous cases). Antibody staining was scored semi-quantitatively for both percentage of tumor cells staining (0,1,2,3) and strength of stain (0,1+,2+,3+). Only nuclear staining for CDX2 and TTF-1 was counted as positive.
Results:
All cases were determined to be of true esophageal origin based on clinical history, imaging, gross impressions of the surgeon and pathologist, and histologic and broad immunohistochemical evaluation by a group of GI-subspecialty pathologists. 23 of the 24 cases displayed strong expression of CK7 (95.8%).
Results:
All cases were determined to be of true esophageal origin based on clinical history, imaging, gross impressions of the surgeon and pathologist, and histologic and broad immunohistochemical evaluation by a group of GI-subspecialty pathologists.
23 of the 24 cases displayed strong expression of CK7 (95.8%).
21 of the 24 cases displayed strong nuclear CDX2 expression (87.5%).
Results:
All cases were determined to be of true esophageal origin based on clinical history, imaging, gross impressions of the surgeon and pathologist, and histologic and broad immunohistochemical evaluation by a group of GI-subspecialty pathologists. 23 of the 24 cases displayed strong expression of CK7 (95.8%). 21 of the 24 cases displayed strong nuclear CDX2 expression (87.5%). Contrary to published literature, 3 of the 24 cases displayed strong TTF-1 nuclear expression (12.5%).
Results:
All cases were determined to be of true esophageal origin based on clinical history, imaging, gross impressions of the surgeon and pathologist, and histologic and broad immunohistochemical evaluation by a group of GI-subspecialty pathologists. 23 of the 24 cases displayed strong expression of CK7 (95.8%). 21 of the 24 cases displayed strong nuclear CDX2 expression (87.5%). Contrary to published literature, 3 of the 24 cases displayed strong TTF-1 nuclear expression (12.5%). Of these three, one is a pT1 tumor in a patient with Barrett’s esophagus. These patients were much younger (p=0.00005) and two of the three tumors also lacked expression of CDX2.
Conclusions:
Nuclear expression of TTF-1 is present in a small subset of EAC cases.
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TTF-1 positive EACs occur in younger patients and typically lack expression of CDX2.
These tumors may represent a unique subtype of esophageal cancer with a distinctive antigenic expression pattern.
Finally, caution should be used when diagnosing pulmonary adenocarcinoma invading the esophagus based on TTF-1 staining in isolation.
684 Unexpected TTF-1 Positivity in a Subset of Gastric Adenocarcinomas. S Choi, EE Furth, PJ Zhang. Hospital of the University of Pennsylvania, Philadelphia, PA.
New microRNA Markers Improves Classification of Indeterminate Inflammatory Bowel Disease

J Lin, J Zhang, N Welker, Z Zhao, Y Li, M Bronner.
Indiana University School of Medicine, Indianapolis, IN;
University of Utah and ARUP Laboratories, Salt Lake City, UT.
Background:
Most inflammatory bowel disease can be specifically classified as either ulcerative colitis or Crohn’s disease, but 5-10% of patients have equivocal features placing them into the indeterminate colitis category.
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Most inflammatory bowel disease can be specifically classified as either ulcerative colitis or Crohn's disease, but 5-10% of patients have equivocal features placing them into the indeterminate colitis category.
In our previous study, 5 microRNA biomarkers have been suggested to assist in the classification of indeterminate inflammatory bowel disease.
This study examines whether additional miRNA markers can increase the diagnostic accuracy.
Design:
Fresh frozen colonic mucosa from the distal-most part of the colectomy from 53 patients was used (16 indeterminate colitis, 14 Crohn's disease, 12 ulcerative colitis, and 11 diverticular disease controls). Total RNA extraction and qRT-PCR amplification was performed. Analysis of variance was performed assessing differences among the groups.
Results:
A significant difference in expressions of miR-19b, 23b, 106a, 191, 629, 147b, 194-2, 383, 615, and 1826 was detected between ulcerative colitis and Crohn's disease groups ($P<0.05$).
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Among the 16 indeterminate colitis patients, 11 showed ulcerative colitis-like pattern, one with Crohn's disease-like pattern, and 4 equivocal.
Conclusions:
Ten microRNA analyses systems further provide molecular evidence that most indeterminate colitis is probably ulcerative colitis.
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Ten microRNA analyses systems further provide molecular evidence that most indeterminate colitis is probably ulcerative colitis. MicroRNA study is promised to improve classification of indeterminate inflammatory bowel disease.
678 MiRNA Gene Profiling Sheds Light onto New Putative Tumorigenic Circuitry for Colorectal Cancer Metastases. L Casadei, S Costinean, V Balatti, L Cascione, CM Croce, WL Frankel. The Ohio State University, Columbus, OH.

691 MiR Profiling of Microsatellite Unstable Colorectal Cancer and SSA Reveals Intriguing New Tumorigenic Pathways. S Costinean, L Casadei, V Balatti, L Cascione, CM Croce, WL Frankel. The Ohio State University, Columbus, OH.

692 P53-miR1246-DYRK1A-NFAT – A Novel Tumorigenic Pathway in Microsatellite Unstable Colorectal Cancers with BRAF Mutation. S Costinean, L Casadei, V Balatti, L Cascione, CM Croce, WL Frankel. The Ohio State University, Columbus, OH.

745 MiR Profiling of Tubular Adenomas and Microsatellite Stable Colorectal Cancer Identifies a New Putative Compensatory Mechanism of MiR1246. C Kavran, S Costinean, L Casadei, V Balatti, L Cascione, CM Croce, WL Frankel. Ohio State University Wexner Medical Center, Columbus, OH; OSU Wexner Medical Center, Columbus, OH.
776 Novel microRNA Signatures to Differentiate Ulcerative Colitis from Crohn's Disease: A Genome-Wide Study Using Next Generation Sequencing. J Lin, NC Welker, Z Zhao, Y Li, J Zhang, MP Bronner. Indiana University School of Medicine, Indianapolis, IN; University of Utah and ARUP Laboratories, Salt Lake City, UT.

800 MicroRNA Sequencing Reveals Distinct Expression Profiles in Neuroendocrine Tumors of the Pancreas and Ileum. NC Panarelli, N Renwick, Z Chen, T Tuschi, Y-T Chen. Weill Cornell Medical College, New York, NY; The Rockefeller University, New York, NY.

844 MicroRNA Profiles Distinguish Benign from Malignant Gastric Mucosa and Differentiate Molecular Subtypes of Gastric Adenocarcinoma. AL Treece, DL Duncan, W Tang, DR Morgan, MO Meyers, RL Dominguez, O Speck, ML Gulley. University of North Carolina at Chapel Hill, Chapel Hill, NC; Vanderbilt University, Nashville, TN; Western Regional Hospital, Santa Rosa de Copan, Honduras.

866 MicroRNA Biomarker Differentiates Inflammatory Bowel Disease and Microscopic Colitis. Z Zhao, H Osman, R Watson, I Nalbantoglu, M Bronner, J Lin. Indiana University School of Medicine, Indianapolis, IN; Washington University, St Louis, MO; University of Utah and ARUP Laboratories, Salt Lake City, UT.
BRAF-Mutated Microsatellite Stable (MSS) Colorectal Carcinoma: An Aggressive Adenocarcinoma with Reduced CDX2 and Increased Cytokeratin 7 Immunohistochemical Expression

M Landau, S-F Kuan, S Chiosea, RK Pai.
University of Pittsburgh Medical Center, Pittsburgh, PA.
Background:
Reduced CDX2 and CK20 expression in colorectal carcinoma (CRC) with \textit{BRAF} mutation and high-level microsatellite instability (MSI-H) has been well documented. The immunophenotype of \textit{BRAF}-mutated microsatellite stable (MSS) CRC has not been reported.
Background:
Reduced CDX2 and CK20 expression in colorectal carcinoma (CRC) with BRAF mutation and high-level microsatellite instability (MSI-H) has been well documented. The immunophenotype of BRAF-mutated microsatellite stable (MSS) CRC has not been reported. The aim of this study was to investigate the expression of CDX2, CK20, and CK7 in BRAF-mutated MSS CRC compared to BRAF-mutated MSI-H and BRAF wild-type CRC.
Design:
205 CRCs including 28 BRAF-mutated MSS, 53 BRAF-mutated MSI-H, and 124 BRAF wild-type MSS tumors were analyzed for CDX2, CK7, and CK20 IHC expression.
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Positive CK7 and CK20 staining was defined as any positive staining.
CDX2 was scored semiquantitatively for intensity (0, absent; 1+, weak; 2+, strong) and percent of tumor cells staining.
A modified H score for CDX2 (intensity x percentage of positive cells) was calculated with positive expression defined as >1.
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CDX2 was scored semiquantitatively for intensity (0, absent; 1+, weak; 2+, strong) and percent of tumor cells staining. 
A modified H score for CDX2 (intensity x percentage of positive cells) was calculated with positive expression defined as >1. 
Each tumor was also analyzed for location, stage, and histologic features.
Results:
*BRAF*-mutated MSS CRC displayed reduced CDX2 expression compared to *BRAF* wild-type MSS CRC (75% vs. 94%; mean H-score 98 vs 150, p<0.001).
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CK7 expression was more often identified in *BRAF*-mutated MSS CRC compared to both *BRAF*-mutated MSI-H CRC and *BRAF* wild-type MSS CRC (39% vs. 6% vs. 6%, p=0.0001).
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There was no difference in CK20 expression between *BRAF*-mutated MSS and *BRAF* wild-type MSS CRC.
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There was no difference in CK20 expression between *BRAF*-mutated MSS and *BRAF* wild-type MSS CRC.

In contrast, *BRAF*-mutated MSI-H CRC was less often CK20 positive (70%, p=0.001).
Results:
BRAF-mutated MSS CRC displayed reduced CDX2 expression compared to BRAF wild-type MSS CRC (75% vs. 94%; mean H-score 98 vs 150, p<0.001).
CK7 expression was more often identified in BRAF-mutated MSS CRC compared to both BRAF-mutated MSI-H CRC and BRAF wild-type MSS CRC (39% vs. 6% vs. 6%, p=0.0001).
There was no difference in CK20 expression between BRAF-mutated MSS and BRAF wild-type MSS CRC.
In contrast, BRAF-mutated MSI-H CRC was less often CK20 positive (70%, p=0.001).
BRAF-mutated MSS CRC were more frequently stage IV compared to BRAF-mutated MSI-H CRC and BRAF wild-type MSS CRC (32% vs. 8% vs. 15%, p<0.001).
Conclusions:

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*BRAF*-mutated MSS CRC is an aggressive tumor which often presents with stage IV disease. *BRAF*-mutated MSS CRC displays reduced CDX2 and increased CK7 expression. Given the frequent presentation of *BRAF*-mutated MSS CRC with metastatic disease, our findings have implications for diagnostic immunohistochemistry when attempting to identify the origin of metastatic adenocarcinoma of unknown primary.
Clinicopathologic and Molecular Correlates of Tumor Budding in Colorectal Carcinoma

Mayo Clinic, Rochester, MN.
Background:
Tumor budding has been proposed as a prognostic factor in colorectal carcinoma (CRC) and is being incorporated into routine clinical practice. The correlation of tumor budding to molecular events and specific CRC pathways has not been investigated.
Design:
We retrieved H&E-stained slides from 553 cases of CRC diagnosed between 1986 and 2002 among women enrolled in the Iowa Women’s Health Study.
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We recorded tumor grade, venous, lymphatic and perineural invasion, tumor morphologic patterns, maximum number of tumor infiltrating lymphocytes (TILs) per 40x objective field, and the maximum number of tumor buds per 20x objective field.
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Tumor budding was classified as

- high if $\geq 10$ buds were seen in a 20x objective field
- low if fewer or no buds were identified.
**Design:**
We retrieved H&E-stained slides from 553 cases of CRC diagnosed between 1986 and 2002 among women enrolled in the Iowa Women’s Health Study. We recorded tumor grade, venous, lymphatic and perineural invasion, tumor morphologic patterns, maximum number of tumor infiltrating lymphocytes (TILs) per 40x objective field, and the maximum number of tumor buds per 20x objective field. Tumor budding was classified as high if ≥10 buds were seen in a 20x objective field and low if fewer or no buds were identified. Each patient had been previously characterized for clinical parameters including smoking history, treatment with chemotherapy, tumor stage, cancer-specific survival and for molecular alterations including microsatellite instability (MSI), *KRAS* and *BRAF* mutation status, and CpG island methylation (CIMP) status. Histologic review was done blinded to all clinical and molecular data.
Results:
High tumor budding (HTB) was identified in 180 cases (32.5%) and 83% of these cases were MSS compared to 68% for cases with low budding (p=0.003).
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High tumor budding (HTB) was identified in 180 cases (32.5%) and 83% of these cases were MSS compared to 68% for cases with low budding (p=0.003). Increasing numbers of TILs were inversely related to tumor budding. MSI-high cases featured a median of 3 tumor buds compared to a median of 6 tumor buds for cases which were MSS or MSI-L. Tumors with HTB were also characterized by KRAS mutations (42% vs 28%; p=0.006), advanced stage (78% vs 54%; p=<0.0001) and receipt of chemotherapy (30% v 20%; p=0.02).
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Tumors with HTB were also characterized by KRAS mutations (42% vs 28%; p=0.006), advanced stage (78% vs 54%; p=<0.0001) and receipt of chemotherapy (30% vs 20%; p=0.02).
There was no correlation between tumor budding and BRAF mutation or CIMP status.
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Kaplan-Meier survival analysis revealed HTB was associated with a worse cancer-specific survival.
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There was no correlation between tumor budding and *BRAF* mutation or CIMP status.
Kaplan-Meier survival analysis revealed HTB was associated with a worse cancer-specific survival.
On multivariate analysis, including all evaluated parameters, HTB was associated with a greater than 2-fold risk of cancer-specific mortality (adjusted HR =2.11, 95% CI 1.42-3.12).
Conclusions:
In this cohort of women with CRC, tumor budding is a marker of aggressive tumor biology, strongly associated with advanced stage and also with MSS, KRAS mutations and more cancer related events.
High tumor budding is a predictor of cancer-specific survival.
748 Tumour Budding Is a Risk Factor of Lymph Node and Distant Metastasis in Malignant Colorectal Polyps with Submucosal Invasive (T1) Carcinoma. S Khutti, S Ligato. Hartford Hospital, Hartford, CT.

773 Tumor Budding in Colorectal Cancer Is Associated with a Decreased Local Tumor and Regional Lymph Node Immunologic Response HH Li-Chang, N Assarzadegan, DE Messenger, A Grin, S Hafezi-Bakhtiari, CJ Howlett, H El-Zimaity, R Kirsch. University of British Columbia, Vancouver, BC, Canada; University of Toronto, Toronto, ON, Canada; Gloucestershire Royal Hospitals NHS Foundation Trust, Gloucester, United Kingdom; Western University, London, ON, Canada.

788 Tumor Budding in Post-Neoadjuvant Chemoradiotherapy Colorectal Cancer AJ McCarthy, AC Rogers, JMP Hyland, R O’Connell, DC Winter, D Gibbons, K Sheahan. Centre for Colorectal Disease, St. Vincent’s University Hospital University College Dublin, Dublin 4, Ireland.
840 Impact of Peritumoral and Intratumoral Budding in Esophageal Adenocarcinomas

S Thies, J Slotta-Huspenina, I Zlobec, VH Koelzer, D Kroell, CA Seiler, M Feith, R Langer. University of Bern, Bern, Switzerland; Technische Universität München, München, Germany; University Hospital Bern, Bern, Switzerland.

845 Clinicopathological Significance of Tumor Budding in Early Gastroesophageal Adenocarcinoma

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A New Regression Grading Proposal for Neoadjuvant-Treated Colorectal Cancer: The Regression Scale

S Serra, P Gill, LM Wang, R Chetty.
University Health Network, Toronto, Canada;
John Radcliffe Hospital, Oxford, United Kingdom.
Background:
The aim of the study was to evaluate the level of concordance among gastrointestinal pathologists using a new regression grading system.
Design:
An international study group proposed that colorectal cancer regression of the entire tumor bed be assessed by using a composite scoring system:
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Each slide/section from the tumour bed should be assessed and given a score as per the following criteria:
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Each slide/section from the tumor bed should be assessed and given a score as per the following criteria:
Score 0: complete pathological regression (no tumor present)
Score 1: little tumor present
Score 2: tumor and regression changes/fibrosis in approximately equal proportion.
Score 3: tumor dominates the slide or no regression
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The individual slide scores are added and the sum divided by the total number of slides (a minimum of 5) from the tumor bed.
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**Score 3:** tumour dominates the slide or no regression
The individual slide scores are added and the sum divided by the total number of slides (a minimum of 5) from the tumor bed. This yields an average score (a cumulative or composite score) that is reflective of the status of the entire tumor bed.
The minimum score per slide is 0 and the maximum 3.
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The minimum score per slide is 0 and the maximum 3. Similarly, when a final cumulative score is obtained for a case, the score will lie on a regression scale from 0 to 3. Five to 6 representative slides were selected from 6 cases of colorectal cancer treated with long-course neoadjuvant chemoradiation. The slides were scanned with a whole-slide scanner generating dynamic digitized images.
The minimum score per slide is 0 and the maximum 3. Similarly, when a final cumulative score is obtained for a case, the score will lie on a regression scale from 0 to 3. Five to 6 representative slides were selected from 6 cases of colorectal cancer treated with long-course neoadjuvant chemoradiation. The slides were scanned with a whole-slide scanner generating dynamic digitized images. The slides were scored at two separate times and intra-observer and inter-observer variability using Kendall’s coefficient of concordance (KCC) were calculated.
Results:
The overall (KCC) Kendall’s Coefficient of Concordance for first round was 0.939571 (p= 0.0000).
KCC for second round was 0.914579 (p= 0.0000).
Conclusions:
Although the exact additive numerical score between pathologists in each slide of each case resulted in poor $k$ stats (largely due to technical issues with the scanned slides and minor variation of figures), the overall level of concordance as measured by the KCC was excellent because of close placement of the case on the regression scale.
Conclusions:
Although the exact additive numerical score between pathologists in each slide of each case resulted in poor $k$ stats (largely due to technical issues with the scanned slides and minor variation of figures), the overall level of concordance as measured by the KCC was excellent because of close placement of the case on the regression scale.
The advantages of this system is that it allows for objective assessment of the entire tumor bed and provides a numerical value that can be plotted on a scale indicating the degree of regression thus providing the oncologist with an overall assessment of the tumor bed.
That's all Folks!
Prevalence and Concordance of Subtypes of Dysplasia in Patients with Barrett’s Esophagus-Associated Adenocarcinoma

AT Agoston, A Srivastava, Y Zheng, R Bueno, RD Odze. Brigham and Women’s Hospital, Boston, MA.

Background: Adenocarcinoma develops in Barrett’s esophagus (BE) via a metaplasia-dysplasia-carcinoma pathway. Two types of dysplasia have been previously identified termed “intestinal” (INT) and “foveolar” (FOV) (i.e. gastric), but the prevalence, significance, and relationship to carcinoma subtypes remain unknown. The aim of this study was to determine the prevalence, and types, of dysplasia in a consecutive series of non-neoadjuvant treated BE-associated adenocarcinomas (BEAd), and to evaluate potential relationships between dysplasia and carcinoma subtypes in order to identify possible multiple pathways of carcinogenesis.

Design: The study group consisted of 156 consecutive patients with BEAd resected without neoadjuvant chemoradiation, at the Brigham and Women’s Hospital (mean age: 66; 83% male). The types, prevalence, and percentage of dysplasia subtypes (INT, FOV, serrated) were evaluated in background mucosa, and correlated with the types of invasive carcinoma. Both dysplasia and adenocarcinoma were defined as “pure” or “predominantly” INT or FOV if ≥90% or >50%, respectively, of the cells showed INT or FOV differentiation.

Results: 122/156 patients (78%) showed dysplasia in background mucosa. The dysplasia was purely INT in 26%, predominantly INT in 34%, purely FOV in 8%, predominantly FOV in 11%, and mixed (≤50% INT and FOV) in 55% of cases. Only one case showed focal serrated dysplasia. Of the 156 carcinomas, 15% showed pure INT, 22% showed predominantly INT, 2.6% showed pure FOV, 5.8% showed predominantly FOV, and 72% showed mixed pattern INT and FOV differentiation. Patients with pure INT dysplasia were significantly associated with pure INT carcinomas (OR=4.74, p=0.002), patients with pure FOV dysplasia were associated with predominantly FOV carcinomas (OR=24.2, p=0.001), and patients with mixed differentiation dysplasia were associated with mixed differentiation carcinomas (OR=6.25, p<0.001).

Conclusions: Intestinal, FOV, or mixed INT/FOV are the most common types of dysplasia in BE. In general, the type of dysplasia correlates with type of carcinoma. This data suggests that multiple distinct pathways of carcinogenesis may occur in BE, and that genetic alterations leading to divergent differentiation may occur early in the pathogenesis of disease, at the dysplasia stage.
Abundance of IgG4-Positive Plasma Cells in Post-Neoadjuvant Ulcers of Gastroesophageal Carcinomas

I Genco, B Saka, S Balci, K Bradley, AB Farriss, A Khosroshahi, B El-Rayes, A Krasinskas, C Staley, D Kooby, S Maithel, V Adsay. Emory, Atlanta, GA.

Background: Abundance of IgG4-positive plasma cells is regarded as a characteristic of specific immunologic entity, originally described as “autoimmune pancreatitis”, and now expanded to include primary idiopathic inflammatory-sclerotic disorders, under the heading of IgG4-Related Disease.

Design: Plasma cell-rich ulcers of 16 post-neoadjuvant resections for gastroesophageal carcinomas (14 adenocarcinomas, 2 squamous cell carcinomas) were immunohistochemically stained for IgG4 and IgG. Eleven patients had chemoradiation therapy, 1 had photodynamic therapy and 1 had radiation therapy. The details of therapy was unknown for 3 patients.

Results: Thirteen of sixteen cases analyzed showed abundant IgG4-positive plasma cells that may have otherwise qualified as IgG4-Related Disease based on the number of positive cells, if the underlying condition was not known. The average age of the patients with abundant IgG4-positive plasma cells was 60, and the patients were 9 males and 4 females. Although only one had history of autoimmune conditions composed of primary biliary cirrhosis, Raynaud’s and Sjogren’s syndromes; all 13 had more than 50 IgG4-positive plasma cells counted in more 3 high power field [the criterion defined by Dhall et al to distinguish autoimmune pancreatitis from other forms of pancreatitis, Hum Pathol. 2010;41(5):643-52]. The mean number of IgG4-positive plasma cells in these cases was 146. Furthermore, IgG4-positive plasma cells/IgG-positive plasma cells ratio of >40% (the cut-off widely used to define IgG4-related sclerosing disorders) was noted in 5 cases, and an additional 3 cases showed a ratio of 38%, 38% and 39%. Average ratio was 40% (range, 23% - 68%).

Conclusions: Abundant IgG4-positive plasma cells can occur as a secondary, iatrogenically-induced phenomenon, and therefore should not be regarded as sole criterion for IgG4-Related Disease. The mechanisms of recruitment of abundant IgG4-positive plasma cells to the ulcers induced by chemo-radiotherapy may shed new light both to the mechanisms of IgG4-positive plasma cell-rich disorders as well as to immunologic processes involved in tumor response to therapy.
A Clinicopathological and Molecular Appraisal of a Large Series of Traditional Serrated Adenomas

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Background: The traditional serrated adenoma (TSA) is a precursor of the serrated neoplasia pathway and among the least common of colorectal polyps. The biology of the TSA remains unclear. Herein we provide detailed clinicopathological and molecular data on the largest series of TSAs so far reported.

Design: A total of 166 TSAs were collected retrospectively from Envoi Pathology and centrally reviewed using strict diagnostic criteria. All were assessed for the common BRAF and KRAS mutations and for the CpG island methylator phenotype (CIMP). A subset (52) have been tested for immunohistochemical markers, including MLH1 and B-catenin.

Results: The clinicopathological and molecular features of the TSAs are provided in Table 1.

<table>
<thead>
<tr>
<th>Mutation Status</th>
<th>Total number</th>
<th>Age</th>
<th>Female</th>
<th>Proximal location</th>
<th>CIMP high</th>
<th>MLH1 loss</th>
<th>Nuclear B-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF mutant</td>
<td>106 (63.9)</td>
<td>65.8</td>
<td>54 (50.9)</td>
<td>43 (40.6)</td>
<td>69 (65.1)</td>
<td>0.29</td>
<td>1/29 (3.4)</td>
</tr>
<tr>
<td>KRAS mutant</td>
<td>36 (21.7)</td>
<td>64.9</td>
<td>20 (55.6)</td>
<td>1 (2.8)</td>
<td>6 (16.7)</td>
<td>0.23</td>
<td>4/23 (17.4)</td>
</tr>
<tr>
<td>Wild type</td>
<td>24 (14.5)</td>
<td>61.0</td>
<td>13 (54.2)</td>
<td>6 (25.0)</td>
<td>3 (12.5)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Number in parentheses indicate percentages

A component of SSA was present in 48 (45.3%) of the BRAF mutant TSAs but was never seen associated with KRAS mutant or wild type TSAs. IHC was performed on 29 BRAF mutant and 23 KRAS mutant TSAs, including 14 cases with either high-grade dysplasia (HGD) and/or early cancer. Nuclear B-catenin expression was present in 5 of these 14 cases (1/6 BRAF mutant and 4/8 KRAS mutant).

Conclusions: TSAs are a heterogeneous molecular group of polyps united by similar morphological appearances. The key division is between BRAF mutant and KRAS mutant/wild-type polyps. BRAF mutant TSAs are often proximal, frequently arise from SSAs and are mostly CIMP-H: KRAS mutant TSAs are mostly rectal, have no apparent precursor and are CIMP-low or negative. MLH1 staining was retained in all TSAs tested in this series, thus TSAs appear to give rise to microsatellite stable (MSS) carcinomas regardless of BRAF or KRAS status. As such, a subset are precursors of the aggressive BRAF mutant, MSS carcinomas. Strong wnt pathway activation appears rare in the BRAF mutant cancers, which is divergent from the majority of colorectal carcinoma.
BRAF V600E Immunohistochemistry Is Sensitive and Specific for Mutation Status in Colorectal Adenocarcinoma and Is Valid before and after (Neo)Adjuvant Therapy

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**Background:** BRAF mutation occurs in a range of human neoplasms including colorectal adenocarcinoma (CRC) and causes constitutive activation of the MAPK signaling pathway leading to oncogenesis. In CRC, BRAF mutation is associated with a worse prognosis and decreased responsiveness to EGFR inhibitor therapy. Therefore, BRAF mutation status is of great clinical interest. Immunohistochemistry (IHC) for mutant V600E BRAF is widely available but there are conflicting reports of its sensitivity and specificity, and little is known about its reliability in tissues post-chemo/radiation therapy.

**Design:** We performed IHC for mutant BRAF (clone: VE1, 1:100 [Spring Bioscience, Pleasanton, CA]) on tissue microarrays (TMAs) of 336 tissue cores from 164 cases of CRC. BRAF V600E mutation was present in 42 cases, as determined by PCR. Cases included tissues taken from primary (n=124) and metastatic (n=40) sites, and those collected before (n=109) and after (n=55) chemo/radiation therapy. Two TMAs (6 BRAF mutants and 33 non-BRAF mutants) were used as a validation cohort to establish the most specific staining pattern. The remaining TMAs were independently evaluated for BRAF staining (+/-) by pathologists at three levels of training (attending, fellow, and resident). In 11 cases, pre- and post-chemo/radiation therapy specimens from the same patient were examined.

**Results:** Consensus examination of the validation cohort revealed a specific staining pattern: diffuse cytoplasmic +/- membranous staining of tumor cells with uniform intensity (which varied from weak to strong). Non-specific nuclear staining occurred in a minority of BRAF mutants and, in the absence of the above criteria, was taken as negative. Averaged across all three observers, and including both pre- and post-treatment specimens, this pattern had: 94% sensitivity, 98% specificity, 95% PPV, and 98% NPV. There was 100% agreement between the 11 cases with pre- and post-treatment specimens. Fleiss’ kappa for inter-observer agreement was 0.96. Pitfalls causing misinterpretation included: signet-ring cell morphology (false negative) and limited tissue for evaluation (false positive).

**Conclusions:** Evaluation of BRAF V600E IHC for a specific staining pattern (diffuse and uniform cytoplasmic) is highly sensitive and specific for BRAF mutation. This pattern is reliable in tissues both pre- and post-chemo/radiation therapy. Interpretation is highly concordant between pathologists at all stages of training. These findings support the use of IHC for primary evaluation of BRAF mutational status in CRC.
BRAF V600E IHQ

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817 BRAF Immunostain in 55 Colon Cancer Cases: Small Cores Are as Useful as Whole Sections. RM Roth, H Hampel, CA Arnold, W Marsh, MM Yearsley, WL Frankel. The Ohio State University Wexner Medical Center, Columbus, OH.

854 Immunohistochemical and Molecular Evaluation of BRAF Mutations in Tumors of the Serrated Neoplastic Pathway. A-S Weidner, NC Panarelli, RK Yantiss, CP Vaughn, WS Samowitz, Y-T Chen. Weill Cornell Medical College, New York, NY; University of Utah, Salt Lake City, UT.

864 Sub-Classification of Colorectal Carcinoma: A Single Institution Evaluation of 204 Resections by MMR IHC, MSI PCR and BRAF V600E IHC (VE1). G Zhang, C Lanigan, R Tubbs, TA Bal, B LaFLeur, S Singh. Cleveland Clinic Foundation, Cleveland, OH; Ventana Medical Systems, Inc, Tucson, AZ.
How Reliable Is pN0 H&E Lymph Node Staging in Colon Cancer? Preliminary Results from a Multicenter Study

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Background: Colon Cancer (CC) is the second cause of death from cancer in developed countries. Pathological lymph-node staging (pN) results from the analysis of 2-5μm thick slices stained with H&E of each lymph-node, which represent <2% of the entire node. About 25% of stage I-II patients (pN0) may develop local recurrence or distant metastases within 5 years of surgery. The sensitivity of H&E for detection of “H&E occult metastases” is low and does not identify those patients that could benefit from adjuvant therapy.

Design: The aim of this study was to assess lymph-node staging differences between H&E staining versus molecular lymph-node staging using One-Step Nucleic Acid Amplification (OSNA), in stage I-II CC patients. This observational, multicenter, prospective study included 199 stage I-II CC patients (16 pTis, 38 pT1, 40 pT2, 89 pT3, 16 pT4), selected from 10 hospitals. Fresh lymph node dissection was done within 50 minutes after surgery. A total of 2,817 lymph-nodes were freshly dissected (mean 13; 8-35). Upon size of the lymph node, a central 1 mm section or ½ of the lymph node was analyzed by H&E, and the rest by OSNA. OSNA is an automated molecular diagnostic assay using nucleic acid amplification technology (RT-LAMP) for the detection of CK19 mRNA. The amount of CK19 mRNA copies correlates with the size of metastatic foci (micrometastases >5,000 copies; micrometastases between 250 and 5,000 copies). Lymph-node staging with H&E was compared to the OSNA lymph-node staging results. The pathology report did not include the OSNA staging, which was blind to the clinician. Patient’s follow-up was recoded.

Results: Of the 199 patients, 162 patients were staged pN0 with H&E, while only 89 resulted N0 using OSNA analysis. 73 patients (45.3, 95% CI 35.6 – 55.2%) contained one or few positive lymph nodes. In only 10% of the patients the total tumor load found within the lymph nodes was high.

Conclusions: Molecular lymph-node staging using OSNA is more sensitive than H&E staging. It allows the analysis of the entire lymph node. Most discords among H&E and OSNA results may be due to tissue allocation bias of non-analyzed tumor by H&E. Molecular lymph node staging may help to select stage I-II CC patients that could benefit from adjuvant therapy. Long-term follow-up of these patients will allow determining the prognostic value of metastases detected by molecular methods.

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740 Next Generation Sequencing (NGS) Reveals Novel Mutations in Goblet Cell Carcinoids and Carcinoma Ex Goblet Cell Carcinoids of the Appendix. M Johncilla, M Stachler, NI Lindeman, R Odze, A Srivastava. Brigham and Women’s Hospital, Boston, MA.

776 Novel microRNA Signatures to Differentiate Ulcerative Colitis from Crohn's Disease: A Genome-Wide Study Using Next Generation Sequencing. J Lin, NC Welker, Z Zhao, Y Li, J Zhang, MP Bronner. Indiana University School of Medicine, Indianapolis, IN; University of Utah and ARUP Laboratories, Salt Lake City, UT.