Anatomic pathology Abstracts

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Clinical and molecular analyses of neuroendocrine carcinomas of breast

July 2018—Neuroendocrine breast carcinomas represent a rare subtype of breast cancer. Their definition, prevalence, and prognosis remain controversial, as reported in the literature. The 2012 World Health Organization classification of breast cancer categorizes neuroendocrine carcinomas into three morphologically distinct subtypes: well-differentiated neuroendocrine tumors, poorly differentiated neuroendocrine carcinomas, and invasive breast carcinomas with neuroendocrine differentiation.

The authors conducted a study to gain insight into the clinical, morphologic, phenotypic, and molecular features of 47 neuroendocrine breast carcinomas. They performed targeted next-generation sequencing by an AmpliSeq 22 cancer gene hotspot panel and the Prosigna assay on 42 of 47 and 35 of 47 cases, respectively. The average age at diagnosis was 69 years. All of the tumors were estrogen receptor positive, and the majority expressed progesterone receptor (89 percent), GATA3 (98 percent), FOXA1 (96 percent), and CK8/18 (98 percent). There was an almost equal distribution of luminal A (52 percent) and B (48 percent) carcinomas. Nearly half (49 percent) of the cohort displayed a high risk of recurrence score with the Prosigna test. Patients with a neuroendocrine carcinoma had a shorter disease-free survival compared with those affected by carcinomas of no special type who were matched for age, size, grade, and estrogen-receptor status. No significant differences were observed in terms of overall survival. Stratification of neuroendocrine carcinomas using the 2012 WHO criteria did not reveal statistically significant differences, in terms of progression-free or overall survival, among the categories of well-differentiated neuroendocrine tumors, poorly differentiated neuroendocrine carcinomas, and invasive breast carcinomas with neuroendocrine differentiation. The targeted sequencing analysis found three (seven percent) cases harboring a *PIK3CA* mutation, and *TP53* mutations in three (seven percent) other cases. This study showed that neuroendocrine breast carcinoma is a distinct subtype of luminal carcinoma with a low rate of *PIK3CA* mutations and aggressive clinical behavior. Accurate identification of neuroendocrine differentiation may be useful to better tailor patient adjuvant therapy within luminal carcinomas.

Lavigne M, Menet E, Tille JC, et al. Comprehensive clinical and molecular analyses of neuroendocrine carcinomas of the breast. *Mod Pathol.* 2018;31:68–82.

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Role of immune microenvironment in gastrointestinal stromal tumors

The immune microenvironment is a prognostic factor for various malignancies. The significance of key players in the immune microenvironment in gastrointestinal stromal tumors (GISTs), including tumor-infiltrating lymphocytes (TILs) and expression of programmed death-ligand 1 (PD-L1), indoleamine 2,3-dioxygenase (IDO), and tryptophanyl-tRNA synthetase (WARS), is largely unknown. The authors conducted a study in which they constructed tissue microarrays from pathology files dated 1996 to 2016. Immunohistochemistry for PD-L1, IDO, and WARS was correlated with tumor size, mitoses, and outcomes. TILs expressing CD3, CD4, CD8, FoxP3, and GBP5 were counted. The authors analyzed 129 GISTs. The mean patient age was 62.5 years, and 52 percent of patients were male. Tumor location included 89 stomach (69 percent), 33 small bowel (25.6 percent), and seven other (5.4 percent). Mean tumor size was 5.6 cm, and mean mitoses were 7.2 per 50 high-power field. Nineteen (15 percent) patients developed disease progression to the abdominal wall (n = 8), liver (n = 6), or elsewhere

(n = 5). Median progression-free survival was 56.6 months; five patients died of disease. PD-L1 was positive in 88 of 127 tumor samples (69 percent); 114 of 127 tumors were IDO positive (89.8 percent); and 60 of 127 tumors were positive for WARS (47.2 percent). PD-L1 was associated with increased size ($P = .01$), necrosis ($P = .018$), and mitoses ($P = .006$). Disease progression was not associated with expression of PD-L1 ($P = .44$), IDO ($P = .14$), or WARS ($P = .36$). PD-L1–positive GISTs with CD8+ or CD3+ TILs were significantly smaller than tumors with CD8+ or CD3+ TILs. The authors concluded that PD-L1 expression was associated with increased size and mitoses. High CD8+ or CD3+ TIL counts were associated with decreased PD-L1/IDO+ GIST size. PD-L1 and IDO could be significant in GIST tumor biology, which invites consideration of immunotherapy as a potential treatment option.

Blakely AM, Matoso A, Patil PA, et al. Role of immune microenvironment in gastrointestinal stromal tumors. *Histopathol.* 2018;72:405–413.

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Use of intestinal metaplasia for diagnosis of Barrett esophagus

Barrett esophagus predisposes patients to developing esophageal adenocarcinoma. However, the “global” definition of Barrett esophagus is controversial. Pathologists in most of Europe and the United States require intestinal metaplasia (IM) within columnar-lined mucosa in the tubular esophagus in order to diagnose the condition, whereas pathologists in the United Kingdom and Japan require only the presence of columnar-lined mucosa. To help establish an appropriate definition for Barrett esophagus, the authors evaluated whether IM accompanies esophageal adenocarcinoma, using a U.S. patient cohort. They examined a series of 139 consecutive patients who underwent endoscopic mucosal resections or esophagectomies for esophageal adenocarcinoma at a U.S. tertiary care center. The authors evaluated the resection specimens for the presence (IM+) or absence (IM−) of IM within columnar-lined mucosa. Ninety-seven (70 percent) patients were IM+. Tumors found in IM− patients tended to be advanced at the time of resection (57 percent pT3 or greater, IM−; 31 percent pT3 or greater, IM+; $P = .02$), such that the tumor may have “overgrown” zones of IM. The authors hypothesized that changes as a result of neoadjuvant chemotherapy or radiation might mask pre-existing IM. When evaluating this hypothesis, they found that 34 of 39 of the treatment-naive patients were IM+. Two of the five IM− patients had prior IM+ biopsies, resulting in 92 percent of treatment-naive patients who were IM+. In the U.S. hospital population, columnar-lined mucosa with IM in the tubular esophagus is found in association with esophageal adenocarcinoma in 70 to 92 percent of patients. The authors concluded that based on these data, the U.S. definition of Barrett esophagus should continue to require the presence of IM.

Salimian KJ, Waters KM, Eze O, et al. Definition of Barrett esophagus in the United States: support for retention of a requirement for goblet cells. *Am J Surg Pathol.* 2018;42(2):264–268.

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Use of select IHC panels in classifying high-grade endometrial carcinomas

Histologic subclassification of high-grade endometrial carcinomas can sometimes be a diagnostic challenge when based on histomorphology alone. The authors conducted a study in which they used immunohistochemical markers to determine the immunophenotype in histologically ambiguous high-grade endometrial carcinomas that were initially diagnosed as pure or mixed high-grade endometrioid carcinoma. The intent of the study was to determine the utility of select immunohistochemical panels for classifying these distinct tumor types, while correlating these findings with clinical outcome. For the study, 43 high-grade endometrial carcinoma cases initially classified as pure high-grade endometrioid carcinoma (n = 32), mixed high-grade endometrioid carcinoma/serous carcinoma (n = 9), and mixed high-grade endometrioid carcinoma/clear cell carcinoma (n = 2) were retrospectively stained with a panel of immunostains, including antibodies for p53, p16, estrogen receptor, and mammaglobin. The authors obtained clinical follow-up data and compared stage-to-stage disease outcomes for different tumor types. Based on

aberrant staining for p53 and p16, 17 of 43 (40 percent) of the high-grade endometrial carcinoma cases initially diagnosed as high-grade endometrioid carcinoma were reclassified as serous carcinoma. All 17 cases showed negative staining for mammaglobin, while estrogen receptor was positive in only six (35 percent) cases. The remaining 26 cases of high-grade endometrioid carcinoma showed wild-type staining for p53 in 25 (96 percent) cases and patchy staining for p16 in 20 (77 percent) cases, and they were positive for mammaglobin and estrogen receptor in eight (31 percent) and 19 (73 percent) cases, respectively. This confirmed the initial diagnosis of high-grade endometrioid carcinoma in these cases. In addition, the cases with reclassified serous carcinoma had advanced clinical stages at diagnosis and poorer overall survival on clinical follow-up compared with the remaining 26 high-grade endometrioid carcinoma cases. These results indicate that select immunohistochemical panels, including p53, p16, and mammaglobin, can be helpful in diagnosing cases of histomorphologically ambiguous endometrial carcinomas and can help guide appropriate therapeutic options for such patients.

Hu S, Hinson JL, Matnani R, et al. Are the uterine serous carcinomas underdiagnosed? Histomorphologic and immunohistochemical correlates and clinical follow up in high-grade endometrial carcinomas initially diagnosed as high-grade endometrioid carcinoma. *Mod Pathol.* 2018;31:358–364.

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A clinicopathologic study of aberrant Pax-8 expression in mesothelioma

Serous ovarian neoplasms can overlap morphologically with peritoneal mesothelial proliferations, including well-differentiated papillary mesothelioma and malignant epithelioid mesothelioma. Accurate histologic classification of these neoplasms is important for clinical management. The Pax-8 protein is commonly used for differentiating peritoneal malignant epithelioid mesothelioma (MM) from serous carcinoma, but the diagnostic value of Pax-8 for distinguishing well-differentiated papillary mesothelioma (WDPM) from borderline or low-grade serous tumors is unknown. The authors used immunohistochemical staining to assess Pax-8 expression in 33 WDPMs, 34 peritoneal MMs, 48 pleural MMs, 11 adenomatoid tumors, five peritoneal inclusion cysts, and 51 benign/reactive mesothelium specimens. Staining was noted in 20 (61 percent) WDPMs, with 17 showing strong and diffuse nuclear staining and three patchy/focal staining. Calretinin was expressed in 33 (100 percent) cases, and focal BerEP4 staining was noted in two of 29 (seven percent) cases. In contrast, four (12 percent) peritoneal MMs were Pax-8 positive (three diffuse and one focal staining). All adenomatoid tumors and peritoneal inclusion cysts were negative for Pax-8. Of the 48 pleural MM cases, two (four percent) showed focal weak to moderate nuclear labeling for Pax-8, and two (four percent) cases of reactive mesothelium demonstrated focal and scattered Pax-8 staining. The authors concluded that Pax-8 appears to be a useful marker for distinguishing MM from gynecologic malignancies but is not reliable for distinguishing WDPM from borderline or low-grade gynecologic lesions.

Xing D, Banet N, Sharma R, et al. Aberrant Pax-8 expression in well-differentiated papillary mesothelioma and malignant mesothelioma of the peritoneum: a clinicopathologic study. *Hum Pathol.* 2018;72:160–166.

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Association of HER2 ITH with incomplete response to anti-HER2 neoadjuvant chemotherapy in breast carcinoma

Anti-HER2 neoadjuvant chemotherapy has been widely used in HER2-positive breast cancer patients, yet pathologic complete response is achieved in only 40 to 50 percent of patients. The authors conducted a study to investigate the association of HER2 intratumoral heterogeneity (ITH) with response to anti-HER2 neoadjuvant chemotherapy. They assessed HER2 ITH on whole tissue sections of pretreatment samples from a cohort of 64 invasive breast carcinoma cases originally considered positive for HER2 and treated with anti-HER2 neoadjuvant chemotherapy. They simultaneously evaluated HER2 gene signal and protein expression using a single-slide dual

assay, designated a HER2 gene-protein assay. The HER2 gene-protein assay was also used on surgical resection tissue from 25 cases with incomplete therapeutic response. Nineteen of 64 (30 percent) cases showed HER2 ITH. Significantly more cases with HER2 ITH were found in the incomplete response group (56 percent; 14 of 25) than in the pathologic complete response group (13 percent; five of 39). Patients in whom ITH was not detectable by gene-protein assay had a 76 percent pathologic complete response outcome (34 of 45), as compared with a 26 percent outcome (five of 19) for those with detectable ITH. Multivariate analysis demonstrated that HER2 ITH, progesterone receptor positivity, and relatively low HER2/chromosome 17 centromere ratio are significantly associated with incomplete response. HER2 ITH analyses conducted with the gene-protein assay method revealed that HER2 ITH is an independent factor, predicting incomplete response to anti-HER2 neoadjuvant chemotherapy.

Hou Y, Nitta H, Wei L, et al. HER2 intratumoral heterogeneity is independently associated with incomplete response to anti-HER2 neoadjuvant chemotherapy in HER2-positive breast carcinoma. *Breast Cancer Res Treat.* 2017;166(2):447–457.

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Use of dual staining to distinguish colorectal carcinomas from other tumors

Small sample size limits the number of immunostains that may be attempted in colorectal carcinoma biopsy specimens. The authors investigated the utility of dual stain with special AT-rich sequence binding protein 2 (SATB2) or caudal-type homeobox 2 (CDX2) and cytokeratin 20 (CK20) or villin in identifying colorectal carcinoma (CRC). They built tissue microarrays with 222 CRCs and 375 other carcinomas and performed dual stain, pairing the nuclear stains CDX2 or SATB2 with CK20 or villin. The authors found that all four single stains showed excellent sensitivity (93–99 percent) but variable specificity (56–88 percent) for CRC. The four dual stains also showed excellent sensitivity (90–96 percent) and much improved specificity (88–98 percent) when compared with the single stains. SATB2 dual stain with CK20 or villin showed a higher specificity than CDX2 dual stain with CK20 or villin and comparable sensitivity. SATB2 dual stain demonstrated the greatest potential clinical utility in identifying CRC and was superior to CDX2 dual stain. More importantly, SATB2 dual stain could be helpful for specimens with limited tissue or having a nonclassic staining pattern.

Li Z, Rock JB, Roth R, et al. Dual stain with SATB2 and CK20/villin is useful to distinguish colorectal carcinomas from other tumors. *Am J Clin Pathol.* 2018;149:241–246.

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Relationship between *KRAS* mutation and implants of serous borderline tumor

In contrast to noninvasive extraovarian implants, invasive implants of ovarian serous borderline tumor/atypical proliferative serous tumor are associated with adverse outcome and have been reclassified as low-grade serous carcinoma. Mutations of *KRAS* or *BRAF*, or both, have been reported in up to 50 percent of serous borderline tumor/atypical proliferative serous tumors. The authors investigated *KRAS* and *BRAF* mutation frequencies in the two types of implants of serous borderline tumor/atypical proliferative serous tumor in correlation with clinical outcome. They included in the study 42 implants of serous borderline tumor from 39 patients (invasive implants/low-grade serous carcinoma, n = 20; noninvasive implants, n = 22). *KRAS* mutation was found in 12 of 20 (60 percent) invasive implants and three of 22 (14 percent) noninvasive implants. *BRAF* V600E mutation was found in one of 22 (five percent) noninvasive implants and no invasive implants. Invasive implants were more frequently associated with higher stage disease. Nine of 14 (64 percent) patients with *KRAS* mutation were found to have stage IIIC disease, while five of 24 (20 percent) patients without the mutation had stage IIIC disease. Patients with

invasive implants had higher recurrence rates compared to those with noninvasive implants (60 versus 14 percent, $P = .0003$, log-rank test) and worse disease-specific survival ($P = .0008$, log-rank test). Regardless of histological subtypes, patients with *KRAS* mutation-positive implants had significantly higher recurrence rates than those without the mutation (71 versus 21 percent, $P = .0021$, log-rank test) and unfavorable disease-specific survival ($P = .0104$, log-rank test). The authors concluded that compared to those with noninvasive implants, patients with invasive implants present with higher stage disease, higher recurrence rates, and worse survival rates. *KRAS* mutation, but not *BRAF* V600E mutation, is significantly associated with invasive implants of serous borderline tumor. Regardless of the histological subtypes of the implants, *KRAS* mutation is a significant prognostic indicator for high risk of tumor recurrence and worse disease-specific survival.

Zuo T, Wong S, Buza N, et al. *KRAS* mutation of extraovarian implants of serous borderline tumor: prognostic indicator for adverse clinical outcome. *Mod Pathol.* 2018;31:350–357.

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Tumor-infiltrating lymphocytes and prognosis in breast cancer

Tumor-infiltrating lymphocytes are predictive for response to neoadjuvant chemotherapy in triple-negative breast cancer and HER2-positive breast cancer. However, their role in luminal breast cancer and the effect of tumor-infiltrating lymphocytes (TILs) on prognosis in all subtypes is less clear. The authors conducted a study in which they assessed the relevance of TILs for chemotherapy response and prognosis in patients with triple-negative breast cancer, HER2-positive breast cancer, and luminal HER2-negative breast cancer. The study included primary breast cancer patients who were treated with neoadjuvant combination chemotherapy and who were from six randomized trials conducted by the German Breast Cancer Group. Pretherapeutic core biopsies from 3,771 patients who participated in these studies were assessed for the number of stromal TILs by standardized methods according to the guidelines of the International TILs Working Group. TILs were analyzed as a continuous parameter and in predefined groups of low (zero to 10 percent immune cells in stromal tissue within the tumor), intermediate (11–59 percent), and high (60 percent or more). The authors used these data in univariable and multivariable statistical models to assess the association between TIL concentration and pathologic complete response in all patients and between the amount of TILs and disease-free survival and overall survival in 2,560 patients from five of the six clinical trial cohorts. In the luminal HER2-negative breast cancer subtype, a pathologic complete response was achieved in 45 of 759 (six percent) patients with low TILs, 48 of 435 (11 percent) with intermediate TILs, and 49 of 172 (28 percent) with high TILs. In the HER2-positive subtype, pathologic complete response was observed in 194 of 605 (32 percent) patients with low TILs, 198 of 512 (39 percent) with intermediate TILs, and 127 of 262 (48 percent) with high TILs. Finally, in the triple-negative breast cancer subtype, pathologic complete response was achieved in 80 of 260 (31 percent) patients with low TILs, 117 of 373 (31 percent) with intermediate TILs, and 136 of 273 (50 percent) with high TILs ($P < .0001$ for each subtype, $\chi 2$ test for trend). In the univariable analysis, a 10 percent increase in TILs was associated with longer disease-free survival in triple-negative breast cancer (hazard ratio [HR], 0.93; 95 percent confidence interval [CI], 0.87–0.98; $P = .011$) and HER2-positive breast cancer (HR, 0.94; 95 percent CI, 0.89–0.99; $P = .017$) but not in luminal HER2-negative tumors (HR, 1.02; 95 percent CI, 0.96–1.09; $P = .46$). The increase in TILs was also associated with longer overall survival in triple-negative breast cancer (HR, 0.92; 95 percent CI, 0.86–0.99; $P = .032$) but had no association in HER2-positive breast cancer (HR, 0.94; 95 percent CI, 0.86–1.02; $P = .11$) and was associated with shorter overall survival in luminal HER2-negative tumors (HR, 1.10; 95 percent CI, 1.02–1.19; $P = .011$). Increased TIL concentration predicted response to neoadjuvant chemotherapy in all molecular subtypes assessed and was associated with a survival benefit in HER2-positive and triple-negative breast cancer. By contrast, increased TILs were an adverse prognostic factor for survival in luminal HER2-negative breast cancer, suggesting a different biology for the immunological infiltrate in this subtype. The authors' data support the hypothesis that breast cancer is immunogenic and might be targetable by immune-modulating therapies. In light of the results for luminal breast cancer, additional research investigating the interaction of the immune system with different types of endocrine therapy is warranted.

Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol.* 2018;19:40–50.

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