Anatomic pathology selected abstracts

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Analysis of *ZC3H7B-BCOR* high-grade endometrial stromal sarcomas

October 2018—High-grade endometrial stromal sarcoma likely encompasses underrecognized tumors harboring genetic abnormalities besides *YWHAE–NUTM2* fusion. Triggered by three initial endometrial stromal sarcomas with *ZC3H7B–BCOR* fusion characterized by high-grade morphology and aggressive clinical behavior, the authors investigated the clinicopathologic features of this genetic subset by expanding the analysis to 17 such tumors. All of the tumors occurred in women who were a median age of 54 (range, 28–71) years. The tumors were predominantly based in the endomyometrium and demonstrated tongue-like or pushing myometrial invasion, or both. Most were uniformly cellular and displayed haphazard fascicles of spindle cells with mild to moderate nuclear atypia. Myxoid matrix was seen in 14 of 17 (82 percent) tumors, and collagen plaques were seen in eight (47 percent). The mitotic index was 10 or more mitotic figures per 10 high-power fields (HPFs) in 14 of 17 (82 percent) tumors, with a median of 14.5 mitotic figures per 10 HPFs. No foci of conventional or variant low-grade endometrial stromal sarcoma were seen. All tumors expressed CD10 with only limited or absent desmin, SMA, or h-caldesmon staining. Estrogen receptor and progesterone receptor expression in more than five percent of cells was seen in four of 12 (33 percent) tumors. Diffuse cyclin D1 and *BCOR* immunoreactivity were present in seven of eight (88 percent) and seven of 14 (50 percent) tumors, respectively. FISH or targeted RNA sequencing confirmed *ZC3H7B–BCOR* fusion in all tumors, including four and two previously diagnosed as myxoid leiomyosarcoma and undifferentiated uterine sarcoma, respectively. Limited clinical data suggest that patients present at higher stage and have worse prognosis compared with published outcomes in low-grade endometrial stromal sarcoma. Tumors with *ZC3H7B–BCOR* fusion constitute a distinct group of endometrial stromal sarcomas with high-grade morphology that should be distinguished from other uterine mesenchymal neoplasms that may demonstrate myxoid morphology.

Lewis N, Soslow RA, Delair DF, et al. *ZC3H7B-BCOR* high-grade endometrial stromal sarcomas: a report of 17 cases of a newly defined entity. *Mod Pathol.* 2018;31:674–684.

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Single-cell heterogeneity in ductal carcinoma in situ of the breast

Heterogeneous patterns of mutations and RNA expression have been well documented in invasive cancers. However, technological challenges have limited the ability to study the heterogeneity of protein expression. This is particularly true for pre-invasive lesions such as ductal carcinoma in situ of the breast. The authors conducted a study in which cell-level heterogeneity in ductal carcinoma in situ was analyzed in a single 5-µm tissue section using a multiplexed immunofluorescence analysis of the disease-related markers EGFR, HER2, HER4, S6, pmTOR, CD44v6, SLC7A5, and CD10, CD4, CD8, and CD20, plus pan-cytokeratin, pan-cadherin, DAPI, and Na+K+ATPase for cell segmentation. Expression was quantified at the cell level using a single-cell segmentation algorithm. K-means clustering was used to determine co-expression patterns of epithelial cell markers and immune markers. The authors documented the presence of epithelial cell heterogeneity within ducts, between ducts, and between patients with ductal carcinoma in situ. They found moderate heterogeneity in a distribution of eight clusters within each duct (average Shannon index, 0.76; range, 0–1.61). Within each patient, the average Shannon index across all ducts ranged from 0.33 to 1.02 (standard deviation, 0.09–0.38). Because the distribution of clusters within ducts was uneven, the analysis of eight ducts might be sufficient to represent all the clusters—that is, within- and between-duct heterogeneity. The pattern of epithelial cell clustering was associated with the presence and type of

immune infiltrates, indicating a complex interaction between the epithelial tumor and immune system for each patient. This analysis provides evidence that simultaneous analysis of both the epithelial and immune/stromal components might be necessary to understand the complex milieu in ductal carcinoma in situ lesions.

Gerdes MJ, Gökmen-Polar Y, Sui Y, et al. Single-cell heterogeneity in ductal carcinoma in situ of breast. *Mod Pathol.* 2018;31:406–417.

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Ultrasensitive automated RNA ISH for detecting B-cell clonality in tissue biopsies

Assessment of B-cell clonality is a critical component of the evaluation of suspected lymphoproliferative disorders, but analysis from formalin-fixed, paraffin-embedded tissues can be challenging if fresh tissue is not available for flow cytometry. Immunohistochemical and conventional bright field in situ hybridization stains for kappa and lambda are effective for evaluating plasma cells but are often insufficiently sensitive to detect the much lower abundance of light chains present in B cells. The authors described an ultrasensitive RNA in situ hybridization assay that has been adapted for use on an automated immunohistochemistry platform and compared the results with flow cytometry in 203 consecutive tissues and 104 consecutive bone marrows. Overall, RNA in situ hybridization identified light chain-restricted B cells in 85 (42 percent) of the 203 tissue biopsies versus 58 (29 percent) by flow cytometry. Within 83 B-cell non-Hodgkin lymphomas, RNA in situ hybridization identified restricted B cells in 74 (89 percent) versus 56 (67 percent) by flow cytometry. B-cell clonality could be evaluated in only 23 (22 percent) of 104 bone marrow cases due to poor RNA preservation, but evaluable cases showed 91 percent concordance with flow cytometry. RNA in situ hybridization allowed for recognition of biclonal/composite lymphomas not identified by flow cytometry and highlighted unexpected findings, such as the co-expression of kappa and lambda RNA in two cases and the presence of lambda light chain RNA in a T lymphoblastic lymphoma. Automated RNA in situ hybridization showed excellent interobserver reproducibility for manual evaluation (average κ, 0.92), and an automated image-analysis system showed high concordance (97 percent) with manual evaluation. Automated RNA in situ hybridization staining, which can be adopted on common immunohistochemistry instruments, allows for the interpretation of clonality in the context of the morphological features in formalin-fixed, paraffin-embedded tissues with a clinical sensitivity similar or superior to flow cytometry.

Guo L, Wang Z, Anderson CM, et al. Ultrasensitive automated RNA in situ hybridization for kappa and lambda light chain mRNA detects B-cell clonality in tissue biopsies with performance comparable or superior to flow cytometry. *Mod Pathol.* 2018;31:385–394.

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Digital image analysis of Ki67 in hot spots: comparison to manual Ki67 and mitotic counts in breast cancer

During pathological examination of breast tumors, proliferative activity is routinely evaluated by a count of mitoses. Adding immunohistochemical stains of Ki67 provides extra prognostic and predictive information. However, the methods for these evaluations suffer from imperfect reproducibility. It is unclear whether Ki67 analysis should be performed in hot spots or the tumor periphery or as an average of the whole tumor section. The authors conducted a study to compare the clinical relevance of mitoses, Ki67, and phosphohistone H3 in two cohorts of primary breast cancer specimens (total n = 294). They evaluated manual and digital image-analysis scores for sensitivity and specificity for luminal B versus A subtype as defined by PAM50 gene-expression assays, high versus low transcriptomic grade, axillary lymph node status, and prognostic value in terms of predicting overall and relapse-free survival. Digital image analysis of Ki67 outperformed the other markers, especially in hot spots. Tumors with high Ki67 expression and high numbers of phosphohistone H3-positive cells had significantly increased hazard ratios for all-cause mortality within 10 years of diagnosis. Replacing manual mitotic counts with digital image analysis of Ki67 in hot spots increased the differences in overall survival between the highest and

lowest histological grades and added significant prognostic information. The authors concluded that digital image analysis of Ki67 in hot spots is the marker of choice for routine analysis of proliferation in breast cancer.

Stålhammar G, Robertson S, Wedlund L, et al. Digital image analysis of Ki67 in hot spots is superior to both manual Ki67 and mitotic counts in breast cancer. *Histopathol.* 2018;72(6):974–989.

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Evaluation of gastric carcinomas with lymphoid stroma

Gastric carcinoma with lymphoid stroma is an uncommon variant enriched for mutually exclusive Epstein-Barr virus positivity and mismatch repair deficiency. The authors evaluated molecular alterations in this morphologically homogeneous subtype and compared them with 295 conventional gastric cancers analyzed in The Cancer Genome Atlas study. They identified 31 study cases and subjected them to in situ hybridization for Epstein-Barr virus (EBV)-encoded RNAs and assessed them for mismatch repair (MMR) status. They performed immunostains for PD-L1, β-catenin, and HER2 and sequenced extracted DNA with a comprehensive cancer panel. Most (76 percent) study patients were older adult men with stage I or II disease. Tumors were classified as EBV+/MMR proficient (MMR-P; n = 7), EBV−/MMR deficient (n = 12), and EBV−/MMR-P (n = 12). EBV−/MMR-P tumors were usually located in the proximal stomach (83 percent) and showed heterogeneous growth patterns with glandular differentiation (83 percent). Tumors in all groups showed numerous tumor-infiltrating lymphocytes and PD-L1 expression and infrequent nuclear β-catenin accumulation (10 percent), and they lacked both membranous HER2 staining and HER2 amplification. EBV−/MMR-deficient tumors showed significantly higher tumor mutation burden ($P = .001$) and *KRAS* alterations (56 percent) compared with EBV−/MMR-P tumors (nine percent; $P = .05$). *TP53* variants were more common among EBV−/MMR-P tumors (82 percent) than among EBV+/MMR-P (zero; P = .01) and EBV−/MMR-deficient (11 percent; $P<.01$) tumors. Alterations in *KRAS, ARID1A, PIK3CA*, and *TP53* followed similar patterns of distribution compared with The Cancer Genome Atlas dataset. The authors concluded that gastric carcinomas with lymphoid stroma show a spectrum of molecular changes and frequent PD-L1 expression, raising the possibility that this subgroup of tumors may be susceptible to checkpoint inhibitors or agents that target receptor tyrosine kinase-mediated signaling, or both.

Hissong E, Ramrattan G, Zhang P, et al. Gastric carcinomas with lymphoid stroma: an evaluation of the histopathologic and molecular features. *Am J Surg Pathol.* 2018;42(4):453–462.

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TP53 mutations in extrauterine high-grade serous carcinomas with two sites of involvement

A previous multicenter study of 67 cases of stage I/II tubo-ovarian high-grade serous carcinoma with complete tubal sampling identified seven cases in which there were only two disease sites, comprising tumor involving opposite adnexa with no extra-adnexal involvement. The authors conducted a study to determine whether such low-stage extrauterine high-grade serous carcinomas with only two sites of involvement, located on opposite adnexa, have identical or different *TP53* mutations in order to investigate their clonal relationship. DNA extracted from both sites of involvement was subjected to *TP53* sequencing (n = 6) or sequencing of one site and mutation confirmation by droplet digital PCR for the other site (n = 1). Of the seven cases analyzed, one had unilateral serous tubal intraepithelial carcinoma with contralateral ovarian high-grade serous carcinoma, three had tubal high-grade serous carcinomas (±serous tubal intraepithelial carcinoma) with contralateral ovarian high-grade serous carcinoma, two had bilateral ovarian high-grade serous carcinomas with normal tubes, and one had bilateral fallopian tube high-grade serous carcinoma with normal ovaries. All seven cases showed identical *TP53* mutations in tumor from both disease sites. Therefore, these rare cases of high-grade serous carcinoma confined to opposite adnexa show clonal identity between the two sites of involvement, indicating unifocal origin and metastasis rather than multifocal origin. These results suggest that serous tubal intraepithelial carcinoma or adnexal high-grade serous carcinoma can metastasize to the contralateral adnexa without peritoneal involvement.

Given the clonal relationship between the two sites, such cases should be considered stage II, with stage I reserved for cases with unilateral and unifocal adnexal involvement. Furthermore, serous tubal intraepithelial carcinoma without invasion should be viewed as constituting a disease site for staging purposes.

Singh N, Faruqi A, Kommoss F, et al. Extrauterine high-grade serous carcinomas with bilateral adnexal involvement as the only two disease sites are clonal based on *tp53* sequencing results: implications for biology, classification, and staging. *Mod Pathol.* 2018;31:652–659.

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Use of *NTRK* fusions to define a novel uterine sarcoma subtype with features of fibrosarcoma

Tropomyosin receptor kinase inhibitors have shown high response rates in patients with tumors harboring *NTRK* fusions. The authors identified four *NTRK* fusion-positive uterine sarcomas that should be distinguished from leiomyosarcoma and undifferentiated uterine sarcoma. *NTRK* rearrangements were detected by FISH or targeted RNA or DNA sequencing in four undifferentiated uterine sarcomas with spindle cell morphology. Because of histologic overlap with leiomyosarcoma, the authors performed tropomyosin receptor kinase A (TrkA) and pan-Trk immunohistochemistry in 97 uterine leiomyosarcomas. They also performed *NTRK1* and *NTRK3* FISH on tumors with TrkA or pan-Trk staining, as well as whole transcriptome RNA sequencing of a leiomyosarcoma with TrkA expression and targeted RNA sequencing of two additional undifferentiated uterine sarcomas. Targeted RNA or DNA sequencing or FISH, or both, showed *TPM3-NTRK1, LMNA-NTRK1, RBPMS-NTRK3,* and *TPR-NTRK1* fusions in the study group. All tumors were composed of fascicles of spindle cells. The mitotic index was seven to 30 mitotic figures per 10 high-power fields. Tumor necrosis was seen in two tumors. Desmin, estrogen receptor, and progesterone receptor were negative in all tumors, while pan-Trk was expressed in all tumors, with concurrent TrkA staining in three of the tumors. TrkA or pan-Trk staining, or both, was also seen in six leiomyosarcomas, but these tumors lacked *NTRK* fusions or alternative isoforms by FISH or whole transcriptome sequencing. No fusions were detected in two undifferentiated uterine sarcomas. *NTRK* fusion-positive uterine spindle cell sarcomas constitute a novel tumor type with features of fibrosarcoma. Patients with these tumors may benefit from Trk inhibition. TrkA and pan-Trk expression in leiomyosarcomas is rare and does not correlate with *NTRK* rearrangement.

Chiang S, Cotzia P, Hyman DM, et al. *NTRK* fusions define a novel uterine sarcoma subtype with features of fibrosarcoma. *Am J Surg Pathol.* 2018;42(6):791–798.

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