Anatomic Pathology Abstracts, 9/17

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Detection of HPV subtypes by mass spectrometry in FFPE tissue specimens

Human papillomavirus infection is a causative agent for approximately five percent of all new cancer cases. The virus is detected in cervical, anal, vaginal, penile, vulvar, and head and neck cancers and has prognostic implications. Therefore, test systems are required to detect high-risk and low-risk HPV subtypes with high specificity and sensitivity in a time-effective and cost-effective manner. The authors conducted a study in which they developed a new mass spectrometry-based test system for detecting HPV infections in formalin-fixed, paraffin-embedded (FFPE) tissue samples. They used a high-throughput matrix-assisted laser desorption ionization time-of-flight mass spectrometry-based assay to genotype 19 HPV types in FFPE tissue specimens (n=46). The results from the assay were compared with the results from two hybridization-based test systems: the HPV 3.5 LCD-array kit and the EuroArrayHPV system. In 36 of 46 (78 percent) tissue samples, an HPV infection could be detected by the mass spectrometry-based HPV assay. In 16 (44 percent) samples, only one HPV subtype was identified; in 20 (56 percent) samples, two to six HPV subtypes were identified. The overall agreement of the three assays was almost perfect (Cohen's kappa value, 0.83). The authors concluded that the mass spectrometry-based assay is highly sensitive, reliable, and cost-effective, and it is a suitable technology for detecting HPV infections in FFPE tissue samples.

Kriegsmann M, Wandernoth P, Lisenko K, et al. Detection of HPV subtypes by mass spectrometry in FFPE tissue specimens: a reliable tool for routine diagnostics. *J Clin Pathol.* 2017;70:417–423.

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PD-L1 in cancer cells and PD-L1+ immune cells in EBV-associated gastric cancer

Cancer cells use PD-L1 to evade antitumor immunity through interaction with programmed cell death protein 1 on T cells. Recent whole genome sequence studies revealed frequent gene amplification of *PD-L1* in Epstein–Barr virus-associated gastric cancer (EBVaGC). To investigate the significance of PD-L1 in cancer cells and their

microenvironment in EBVaGC, the authors studied PD-L1 expression by analysis of a public database and immunohistochemistry with FISH of the *PD-L1* gene. Analysis of the database from The Cancer Genome Atlas also disclosed high expression of PD-L1 in EBVaGC compared with expression in other molecular subtypes of gastric cancer. Expression of PD-L1 was frequently detected in cancer cells of EBVaGC (33 of 96; 34 percent), with infiltration of PD-L1+ immune cells in its stroma (43 of 96; 45 percent). Expression of PD-L1 in cancer cells and PD-L1+ immune cell infiltration in EBVaGC were significantly correlated with diffuse histology according to Lauren's classification and tumor invasion (pT1b or more). As a prognostic indicator, PD-L1 expression in cancer cells correlated with poor outcome in overall survival and disease-specific survival (P=.0498, .007). PD-L1–positive cancers had dense infiltration of PD-L1+ immune cells as well as CD8+ and PD-1+ cells in EBVaGC. FISH analysis of representative samples of the tumor demonstrated gene amplification of *PD-L1* in 11 percent of cases. PD-L1–amplified cells corresponded to PD-L1–positive cells showing high-intensity immunohistochemical staining among cancer cells showing weak or moderate intensities. Taken together, PD-L1 expression in cancer cells and their microenvironment may contribute to the progression of EBVaGC, and gene amplification occurs as clonal evolution during progression. This specific subtype of gastric cancer associated with EBV is potentially a good candidate for immunotherapy targeting the PD-L1/PD-1 axis.

Saito R, Abe H, Kunita A, et al. Overexpression and gene amplification of PD-L1 in cancer cells and PD-L1+ immune cells in Epstein-Barr virus-associated gastric cancer: the prognostic implications. *Mod Pathol.* 2017;30:427–439.

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GIST manifesting as a retroperitoneal tumor: a genetic study of 112 cases

Most gastrointestinal stromal tumors occur in the tubular gastrointestinal tract, but some apparently present outside the GI tract. The authors conducted a study in which they analyzed 112 gastrointestinal stromal tumors (GISTs) located in the retroperitoneum. The tumors occurred in 55 women and 57 men who were a median age of 65 years (range, 21–89 years). On the basis of clinically or histologically detected connections to the GI tract, 15 tumors were considered likely of gastric origin, nine duodenal, and 13 small intestine. The remaining cases were categorized by location as peripancreatic (n=25), pelvic (n=11), mesenteric (n=4), and of unspecified/miscellaneous sites (n=35). The tumors varied in size from 3 to 35 cm (median, 15 cm) and by mitotic rate per 5 mm2, zero to more than 100 (median, 10). Histologically, the tumors apparently arising outside the GI tract had features of intestinal (n=41) and gastric GISTs (n=25); nine cases had indeterminate histology. The histologic variants included spindled, epithelioid, vacuolated, nested, and myxoid potentially simulating other tumors, such as liposarcoma and solitary fibrous tumor. Most GISTs were KIT positive (106 of 112 cases), and the remaining six tumors were DOG1/Ano1 positive. Five cases showed focal nuclear positivity for MDM2. KIT mutations were detected in 42 of 59 cases and PDGFRA mutations in four of 16 KIT wild-type and three of five of the KIT-negative tumors analyzed. One pelvic retroperitoneal GIST was succinate dehydrogenase deficient. All 79 patients were deceased at last follow-up, with a median survival of 14 months and few surviving longer than five years. Only operable versus inoperable tumor was a statistically favorable factor in univariate analysis (P<.01). In multivariate analysis, a mitotic rate greater than 50/5 mm2 was significant for a shorter survival (hazard ratio, 5.25; 95 percent confidence interval, 1.65–16.8; P<.01). The histologic and clinicopathologic similarity of extragastrointestinal retroperitoneal GISTs to GISTs of the GI tract suggests their GI tract origin. The potentially overlapping features of GIST and other retroperitoneal tumors necessitate use of multiple diagnostic markers and molecular genetic studies.

Miettinen M, Felisiak-Golabek A, Wang Z, et al. GIST manifesting as a retroperitoneal tumor: clinicopathologic immunohistochemical and molecular genetic study of 112 cases. *Am J Surg Pathol.* 2017;41(5):577–585.

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Male breast cancer precursor lesions: analysis of a breast cancer program

Data regarding breast cancer carcinogenesis in men are limited. The authors conducted a study to describe the presence of precursor lesions adjacent to invasive male breast cancer in order to increase understanding of carcinogenesis in this population. Central pathology review was performed for 1,328 male breast cancer patients, registered in the retrospective joint analysis of the International Male Breast Cancer Program, and included the presence and type of breast cancer precursor lesions. In a subset, invasive breast cancer was compared with the adjacent precursor lesion by immunohistochemistry (n=83) or targeted next-generation sequencing (n=7). The authors correlated the presence of ductal carcinoma in situ with outcome. A substantial proportion (46.2 percent) of patients with invasive breast cancer also had an adjacent precursor lesion, primarily ductal carcinoma in situ (97.9 percent). The presence of lobular carcinoma in situ and columnar cell-like lesions was very low (less than one percent). In the subset of invasive breast cancer cases with adjacent ductal carcinoma in situ (n=83), complete concordance was observed between the estrogen receptor, progesterone receptor, and HER2 status of both components. Next-generation sequencing on a subset of cases with invasive breast cancer and adjacent ductal carcinoma in situ (n=4) showed identical genomic aberrations, including *PIK3CA, GATA3, TP53,* and *MAP2K4* mutations. Next-generation sequencing on a subset of cases with invasive breast cancer and an adjacent columnar cell-like lesion showed genomic concordance in two of three patients. A multivariate Cox model for survival showed a trend in which the presence of ductal carcinoma in situ was associated with better overall survival, particularly in the luminal B HER2+ subgroup. The authors concluded that ductal carcinoma in situ is the most commonly observed precursor lesion in male breast cancer and that its presence seems to be associated with better outcome, particularly in luminal B HER2+ cases. The rate of lobular carcinoma in situ and columnar cell-like lesions adjacent to male breast cancer is very low, but these findings support the role of columnar cell-like lesions as a precursor of male breast cancer.

Doebar SC, Slaets L, Cardoso F, et al. Male breast cancer precursor lesions: analysis of the EORTC 10085/TBCRC/BIG/NABCG International Male Breast Cancer Program. *Mod Pathol.* 2017;30:509–518.

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Vulvar intraepithelial lesions and keratinizing SCC: a comparison

Human papillomavirus-negative keratinizing vulvar cancers and their precursors, differentiated vulvar intraepithelial neoplasia, typically harbor *TP53* mutations. However, atypical verruciform proliferations are also associated with these malignancies, and their pathogenesis is poorly understood. The authors conducted a study in which they compared 11 atypical verruciform lesions, including atypical verruciform hyperplasia, vulvar acanthosis with altered differentiation, and verruciform lichen simplex chronicus, with 14 human papillomavirus-negative keratinizing squamous cell carcinomas. Extracted tissue DNA was subjected to targeted massively parallel sequencing of the exonic regions of 300 genes. Eight (73 percent) and six (55 percent) of 11 atypical verruciform lesions contained mutations in *PIK3CA* and *ARID2*, respectively. No *TP53* mutations were identified. Eleven (79 percent) and five (36 percent) of 14 keratinizing squamous cell carcinomas tested contained *TP53* and *CDKN2A* mutations, respectively. Keratinizing squamous cell carcinomas displayed the majority of copy number variations, with some variations—7p gain and 8p loss—shared by some cases in both groups. One patient developed atypical verruciform lesions with *PIK3CA* mutations followed by a keratinizing carcinoma with mutations in *PIK3CA* and *TP53*. This study segregates atypical verruciform lesions by virtue of a unique genotype (*PIK3CA* mutant/*TP53* wild type) illustrating an example of progression to a *TP53*-mutated keratinizing carcinoma. The findings indicate that although *PIK3CA* mutations are found in fewer than 10 percent of vulvar squamous cell carcinomas, they may be specific for a particular pathway involving atypical verruciform lesions, which could function as either a direct precursor or a risk factor for vulvar squamous cell carcinoma. Given the presence of a molecular signature, the authors propose the term “differentiated exophytic vulvar intraepithelial lesion” for this group. Whether they function as direct precursors to a less common form of squamous cell carcinoma will require further study, but

carcinomas associated with these lesions might warrant testing for *PIK3CA* mutations to address this question.

Watkins JC, Howitt BE, Horowitz NS, et al. Differentiated exophytic vulvar intraepithelial lesions are genetically distinct from keratinizing squamous cell carcinomas and contain mutations in PIK3CA. *Mod Pathol.* 2017;30:448–458.

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Biological relevance of human papillomaviruses in vulvar cancer

The carcinogenic role of high-risk human papillomavirus (HR-HPV) types in the growing subset of vulvar intraepithelial neoplasia and vulvar cancer in young women has been established. However, the number of vulvar cancer cases attributed to HPV is still imprecisely defined. In an attempt to provide a more precise definition of HPV-driven vulvar cancer, the authors performed HPV type-specific E6*I mRNA analyses, available for 20 HR-/possible HR (pHR)-HPV types, on tissue samples from 447 cases of vulvar cancer. They performed HPV DNA genotyping using the SPF10-LiPA25 assay because of its high sensitivity in formalin-fixed, paraffin-embedded tissues. Data on p16INK4a expression was available for comparative analysis via kappa statistics. The use of highly sensitive assays covering the detection of HPV mRNA in a broad spectrum of mucosal HPV types resulted in the detection of viral transcripts in 87 percent of HPV DNA+ vulvar cancers. Overall concordance between HPV mRNA+ and p16INK4a upregulation (strong, diffuse immunostaining in more than 25 percent of tumor cells) was 92 percent (k=.625; 95 percent confidence interval [CI], 0.531–0.719). Among these cases, 83 percent were concordant pairs of HPV mRNA+ and p16INK4a+ and nine percent were concordant pairs of HPV mRNA− and p16INK4a−. These data confirm the biological role of HR-/pHR-HPV types in the vast majority of HPV DNA+ vulvar cancers, resulting in an HPV-attributable fraction of at least 21 percent worldwide. Most HPV DNA+ vulvar cancers were associated with HPV 16 (85 percent), but a causative role for other, less frequently occurring mucosal HPV types (HPV 26, 66, 67, 68, 70, and 73) was also confirmed at the mRNA level. These findings should be taken into consideration for future screening options because HPV-associated vulvar preneoplastic lesions have increased in incidence in younger women and require different treatment than vulvar lesions that develop from rare autoimmune-related mechanisms in older women.

Halec G, Alemany L, Quiros B, et al, on behalf of the HPV VVAP Study Group. Biological relevance of human papillomaviruses in vulvar cancer. *Mod Pathol.* 2017;30:549–562.

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