Anatomic Pathology Selected Abstracts, 6/14

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Utility of triple antibody cocktail intraurothelial neoplasm-3 and AMACR in urothelial CIS and reactive urothelial atypia

Urothelial carcinoma in situ (CIS) is a prognostically and therapeutically significant lesion with considerable morphologic overlap with reactive conditions, especially in the setting of prior therapy. Various markers, including CK20, CD44s, and p53, have been used as an adjunct in making this distinction. However, the utility of these markers in the post-treatment scenario is not fully established. The tumor-associated marker α-methy-lacyl-CoA racemase (AMACR) is expressed in a subset of high-grade urothelial carcinomas but has not been studied in CIS. Therefore, the authors conducted a study to evaluate the immunoreactivity of CK20, CD44s, and p53 as a triple antibody cocktail intraurothelial neoplasm-3 (IUN-3) in distinguishing CIS from its mimics and to compare its utility with AMACR in the diagnosis of CIS. The study involved 135 specimens—seven benign ureters and 128 bladder biopsies (28 reactive, 33 post-therapy reactive, 43 CIS, 24 CIS post-therapy). Immunostaining for p53 (brown, nuclear), CD44s (brown, membranous), and CK20 (red, cytoplasmic and membranous) was performed as a cocktail, and the staining pattern was further classified as malignant (full-thickness CK20 and/or full-thickness p53 with CD44s negativity), reactive/benign (CK20 limited to the umbrella cell layer, p53 negative, and CD44s positivity ranging from basal to full thickness), or indeterminate (CK20 and p53 positive but not full thickness and/or CD44s positive). AMACR staining was performed in 50 cases. Cytoplasmic staining for AMACR was graded as negative (absent to weak focal staining [fewer than five percent of cells]) and positive (five percent or greater). The IUN-3–malignant pattern was observed in 84 percent of cases of CIS without a history of prior therapy and 71 percent of cases of CIS with a history of prior therapy. Cases with post-therapy reactive atypia showed an IUN-3–reactive pattern in 84 percent of cases and IUN-3–indeterminate pattern in 16 percent of cases. The IUN-3–malignant pattern was not identified in any cases. Benign and reactive urothelium, with and without a history of therapy, showed an IUN-3–reactive pattern and negative AMACR staining in all cases. AMACR positivity was observed in 78 percent of nontreated CIS cases and 50 percent of CIS post-therapy cases. In these cases, the IUN-3 cocktail showed an IUN-3–malignant pattern in 83 percent of untreated CIS cases and 88 percent of CIS post-therapy cases. The authors concluded that AMACR positivity is a potentially useful marker of CIS. However, the IUN-3–malignant pattern is a more reliable indicator of CIS compared with AMACR, especially in the post-treatment setting. The simultaneous evaluation of all three markers—p53, CD44s, and CK20—in a single slide in the form of a cocktail is advantageous, especially with small biopsy specimens.

Aron M, Luthringer DJ, McKenney JK, et al. Utility of a triple antibody cocktail intraurothelial neoplasm-3 (IUN-3-CK20/CK44s/p53) and α-methylacyl-CoA racemase (AMACR) in the distinction of urothelial carcinoma in situ (CIS) and reactive urothelial atypia. *Am J Surg Pathol.* 2013;37:1815–1823.

Correspondence information not provided.

Use of cervical mucus to screen for gynecological malignancies

High-grade malignancies are the leading cause of death from gynecological tumors. Unfortunately, no efficient screening method is available for these tumors. The authors reported the results of a pilot study based on the frequency of TP53 mutations in these cancers. Mucus from the cervix of 32 hysterectomy specimens with no grossly visible cervical or serosal involvement were included in the study. TP53 exons 5–9 mutations were screened for mutations using single-strand conformation polymorphism (SSCP). Immunostain for p53 protein was performed in all fallopian tubes and in a sample from the tumors that were identified prospectively. Thirty-two

cases, including 19 malignant and 13 benign, were included. P53 immunostain was positive in only five of the cases—three high-grade malignant tumors and two precancerous lesions (serous tubal intraepithelial lesion or p53 signature) in the fallopian tubes. A TP53 mutation band pattern was detected by SSCP in two of three and two of two cases, respectively. Twenty-seven cases were negative for p53 immunostain, four of which were positive for TP53 mutation by SSCP, including three low-grade malignancies. The authors concluded that the results of this study provide evidence that DNA from precursor lesions of high-grade ovarian, fallopian tube, and endometrial carcinomas can be detected in cervical mucus. Additional studies using different markers in a preoperative setting and large-scale screening studies will determine the utility of using cervical mucus to screen for gynecological malignancies.

Lamzabi I, Buckingham L, Gebrekiristos M, et al. Use of cervical mucus to screen for gynecological malignancies: a pilot study. *Mod Pathol.* 2013;26:1508–1513.

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Relationship between PTEN, DNA mismatch repair, and tumor histotype in endometrial carcinoma

Loss of *PTEN* (phosphatase and tensin homolog) expression and microsatellite instability are two of the more common molecular alterations in endometrial carcinoma. Controversy surrounds whether there is a relationship between these molecular mechanisms. Therefore, a cohort of 187 pure endometrioid and nonendometrioid endometrial carcinomas, carefully characterized as to clinical and pathological features, was examined by the authors for *PTEN* sequence abnormalities and the immunohistochemical expression of PTEN and the DNA mismatch repair proteins MLH1, MSH2, MSH6, and PMS2. MLH1 methylation analysis was performed when tumors had loss of MLH1 protein. Mismatch repair protein loss was more frequent in endometrioid carcinomas compared with nonendometrioid carcinomas, a difference primarily attributable to the presence of MLH1 methylation in a greater proportion of endometrioid tumors. Among the nonendometrioid group, mixed endometrioid/nonendometrioid carcinomas were the histotype that most commonly had loss of mismatch repair protein. In endometrioid tumors, the frequency or PTEN loss measured by immunohistochemistry and mutation did not differ significantly between the mismatch repair protein intact or mismatch repair protein loss groups, suggesting that PTEN loss is independent of mismatch protein repair status in this group. However, in nonendometrioid carcinomas, intact positive PTEN immunohistochemical expression and wild-type *PTEN* were highly associated with retained positive expression of mismatch repair proteins in the tumor. Of relevance to screening endometrial cancers for Lynch syndrome is that an initial PTEN immunohistochemistry determination may be able to replace the use of four mismatch repair immunohistochemical markers in 63 percent of patients with nonendometrioid endometrial carcinoma. Therefore, PTEN immunohistochemistry, combined with tumor histotype, is a useful adjunct in the clinical evaluation of endometrial carcinomas for Lynch syndrome.

Djordjevic B, Barkoh BA, Luthra R, et al. Relationship between PTEN, DNA mismatch repair, and tumor histotype in endometrial carcinoma: retained positive expression of PTEN preferentially identifies sporadic non-endometrioid carcinomas. *Mod Pathol.* 2013;26:1401–1412.

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ZEB1 overexpression associated with E-cadherin and microRNA-200 downregulation

Undifferentiated endometrial carcinomas are very aggressive high-grade endometrial carcinomas that are frequently underrecognized. The authors conducted a study to analyze the molecular alterations underlying the development of these endometrial carcinomas, focusing on those related to dedifferentiation. They assessed a series of 120 tumors: 57 grade 1 and 2 endometrioid endometrial carcinomas, 15 grade 3 endometrioid endometrial carcinomas, 27 endometrial serous carcinomas, and 21 undifferentiated endometrial carcinomas. The

authors found a high frequency of DNA mismatch repair deficiency (38 percent) and moderate rate of p53 overexpression (approximately 33 percent) in undifferentiated carcinomas. In contrast to the characteristic endometrioid phenotype, a dramatic downregulation of E-cadherin expression was noted in the undifferentiated subtype. Quantitative methylation studies dismissed CDH1 promoter hypermethylation as the mechanism responsible for this change in gene expression, while immunohistochemistry revealed that the E-cadherin repressor ZEB1 was frequently overexpressed (62 percent) in undifferentiated endometrial carcinomas. This finding was accompanied by a sharp downregulation in the expression of the miR-200 family of microRNAs, well-known targets of ZEB1. Furthermore, there was enhanced expression of epithelial-to-mesenchymal transition markers in undifferentiated endometrial carcinomas, such as N-cadherin, cytoplasmic p120, and osteonectin. In addition, HMGA2, a regulator of epithelial-to-mesenchymal transition that is expressed in aggressive endometrial tumors, such as endometrial serous carcinomas and carcinosarcomas, was expressed in more than 20 percent of undifferentiated carcinomas. These results suggest that ZEB1 overexpression, associated with E-cadherin and miR-200s downregulation, and the expression of mesenchymal markers might enhance the metastatic potential of undifferentiated endometrial carcinomas, leading to a poor prognosis. Furthermore, these observations suggest that immunohistochemical analysis of E-cadherin and ZEB1 can aid in the differential diagnosis of the more aggressive undifferentiated endometrial carcinomas from grade 3 endometrioid carcinomas.

Romero-Pérez L, López-Garcia MA, Díaz-Martin J, et al. ZEB1 overexpression associated with E-cadherin and microRNA-200 downregulation is characteristic of undifferentiated endometrial carcinoma. *Mod Pathol.* 2013;26:1514–1524.

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Human papillomavirus-associated oral intraepithelial neoplasia

The authors evaluated an unusual subset of oral epithelial dysplasia for the presence of transcriptionally active high-risk human papillomavirus subtypes and to further characterize the histological criteria for this condition. Clinical and followup data were collected and histopathological features documented. Twenty cases were diagnosed as epithelial dysplasia with marked apoptosis of the anterior oral cavity. Immunoperoxidase studies were performed for p16, and in situ hybridization studies were performed for low- and high-risk HPV subtypes. Gender- and site-matched controls of conventional moderate to severe oral epithelial dysplasia were similarly evaluated using immunoperoxidase studies for p16 and in situ hybridization. The number of apoptotic cells for study and control cases was counted at two tissue sites. The evaluation focused on 17 men and three women who were a median age of 56 years. Seventeen lesions were described as white and five as rough or papillary. Thirteen were located on the lateral or ventral tongue, some extending onto the floor of the mouth. Epithelial hyperplasia with marked karyorrhexis and apoptosis were present in all the cases, along with features of conventional oral epithelial dysplasia. A statistically significant number of apoptotic cells were identified in the study cases when compared with controls ($P>0.0001$). Twenty cases were positive for high-risk HPV by in situ hybridization, and all 19 cases evaluated for p16 demonstrated overexpression. Two patients were diagnosed with squamous cell carcinomas and one patient developed recurrent disease. For consistency in nomenclature with HPV-associated lesions of the lower anogenital tract, the authors propose using the term HPV-associated oral intraepithelial neoplasia to characterize a subset of oral intraepithelial dysplasia that occurs mostly on the ventral or lateral tongue of adult males and is positive for high-risk HPV and p16.

Woo SB, Cashman EC, Lerman MA. Human papillomavirus-associated oral intraepithelial neoplasia. *Mod Pathol.* 2013;26:1288–1297.

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Association between tumor-infiltrating lymphocyte grade in

primary melanomas and melanoma-specific survival

Although most hospital-based studies suggest more favorable survival outcomes with tumor-infiltrating lymphocytes present in primary melanomas, it is uncertain whether TILs provide prognostic information beyond melanoma-staging definitions. The authors addressed this issue in an international population-based study of patients with single and multiple primary melanomas. On the basis of the Genes, Environment and Melanoma (GEM) Study, they conducted followup of 2,845 patients diagnosed from 1998 to 2003 with 3,330 invasive primary melanomas centrally reviewed for TIL grade (absent, nonbrisk, or brisk). They examined the odds of TIL grades associated with clinicopathologic features and survival by TIL grade. The authors found that the independent predictors ($P<0.05$) for nonbrisk TIL grade were site, histologic subtype, and Breslow thickness, and for brisk TIL grade were age, site, Breslow thickness, and radial growth phase. Both nonbrisk and brisk TIL grades were associated with lower American Joint Committee on Cancer (AJCC) tumor stage compared with TIL absence (P [trend] <0.001). Death rate as a result of melanoma was 30 percent less with nonbrisk TIL grade (hazard ratio [HR], 0.7; 95 percent confidence interval [CI], 0.5–1.0) and 50 percent less with brisk TIL grade (HR, 0.5; 95 percent CI, 0.3–0.9) relative to TIL absence, adjusted for age, gender, site, and AJCC tumor stage. The authors concluded that at the population level, higher TIL grade of primary melanoma is associated with a lower risk of death as a result of melanoma independent of tumor characteristics used for AJCC tumor stage. TIL grade deserves further prospective investigation to determine whether it should be included in future AJCC staging revisions.

Thomas NE, Busam KJ, From L, et al. Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based Genes, Environment and Melanoma Study. *J Clin Oncol.* 2013;31:4252–4259.

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Study of PEComas of the gastrointestinal tract with evaluation of prognostic parameters

Perivascular epithelioid cell tumors (PEComas) are distinctive mesenchymal neoplasms that typically arise in the retroperitoneum, visceral organs, and abdominopelvic sites and usually show reactivity for melanocytic and smooth muscle markers. Fewer than 20 PEComas of the gastrointestinal tract have been reported, and behavior and criteria for malignancy are incompletely defined. The authors conducted a study to examine the clinicopathologic features of a series of GI PEComas and to evaluate prognostic parameters. Thirty-five PEComas of the GI tract were retrieved from consult and surgical files. Clinical and pathologic features were evaluated, and immunohistochemical analysis was performed. Clinical followup information was obtained from medical records and referring physicians. Nineteen patients were female and 16 male (median age, 45 years; range, 7–70 years). One patient had tuberous sclerosis. Nineteen tumors arose in the colon, 12 in the small bowel, two in the stomach, and one each in the gallbladder and omentum. Median tumor size was 6.2 cm (range, 0.8–22 cm). Three tumors were limited to the mucosa and submucosa, while eight extended to the muscularis propria, 15 to the subserosa/serosa, and eight into the mesentery. The tumors were composed of nests and sheets of usually epithelioid cells with abundant granular eosinophilic to clear cytoplasm surrounded by a delicate capillary vasculature. Thirteen tumors had mixed epithelioid and spindle cell components and two were purely spindled. Sixteen tumors showed marked nuclear atypia. Seventeen tumors contained occasional pleomorphic cells and 12 showed diffuse cellular pleomorphism. The median mitotic rate was 2/10 high-power field (HPF; range, 0–36). Vascular invasion was present in five cases, and 16 tumors showed necrosis. By immunohistochemistry, 23 of 35 tumors were positive for HMB45, 23 of 34 for melan-A, 15 of 25 for MiTF, 20 of 35 for smooth muscle actin, 26 of 35 for desmin, and three of 20 for TFE3. Focal cytoplasmic S100 protein was present in five of 27 cases, while two of 25 cases were positive for KIT, and one case each was positive for epithelial membrane antigen and keratin. Followup information was available for 31 patients (median, 36 months; range, 2–176 months). Thirteen patients had developed metastases (10 liver, three peritoneum, four lymph node, three lung, one bone, one brain, and one adrenal). The authors reported that five patients had died of disease. Metastases were significantly associated with marked atypia, diffuse pleomorphism, and mitoses ≥ 2/10 HPF. The authors concluded that PEComas of the GI tract

occur at similar frequency in female and male patients, most commonly involve the colon, and exhibit variable clinical behavior that ranges from benign lesions to aggressive, high-grade sarcomas. The presence of marked nuclear atypia, diffuse pleomorphism, and mitotic activity are the strongest predictors of malignant behavior.

Doyle LA, Hornick JL, Fletcher CD. PEComa of the gastrointestinal tract: clinicopathologic study of 35 cases with evaluation of prognostic parameters. *Am J Surg Pathol.* 2013;37 (12):1769–1782.

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