Anatomic Pathology Selected Abstracts, 10/13

Anatomic pathology abstracts editors: Michael Cibull, MD, professor and vice chair, Department of Pathology and Laboratory Medicine, University of Kentucky College of Medicine, Lexington; Rouzan Karabakhtsian, MD, attending pathologist, Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; and Thomas Cibull, MD, dermatopathologist, Evanston Hospital, NorthShore University HealthSystem, Evanston, Ill.

Acellular mucin in rectal cancer patients showing pathologic complete response to preoperative chemoradiotherapy

Patients with locally advanced rectal adenocarcinoma who receive preoperative chemoradiotherapy occasionally show acellular mucin in resection specimens that had shown pathologic complete response, but the clinical and prognostic significance of this finding has generated controversy. The authors analyzed data from 217 consecutive patients showing pathologic complete response (pCR) to preoperative chemoradiotherapy (CRT) followed by resection to evaluate the clinicopathologic features and prognostic significance of acellular mucin. Patients were categorized according to the presence of acellular mucin, as identified by pathologic analysis. Clinicopathologic findings and oncologic results were compared. Acellular mucins were identified in 35 (16.1 percent) of 217 pCR patients. They were found predominantly in male patients (20.8 percent versus 9.8 percent; P=.039) and in those with mucinous/signet ring cell differentiation (66.7 percent versus 15.1 percent; P=.008). The presence of acellular mucin was more frequent in patients with a shorter (less than 42 days) CRT-operation interval (22.6 percent versus 10.3 percent; P=.017). With a mean followup of 41 months (range, two to 119 months), the three-year overall survival rate (96.8 percent with mucin versus 95.9 percent without mucin; P=.314) and three-year disease-free survival rate (97 percent with mucin versus 93 percent without mucin; P=.131) did not differ between the groups. The authors concluded that the presence of acellular mucin in rectal cancer patients showing pCR to preoperative CRT is associated with male gender and mucinous differentiation and does not have a significant impact on oncologic outcomes. Acellular mucins are also associated with the CRT-operation interval as a phenomenon of time-dependent response to CRT.

Lim SB, Hong SM, Yu CS, et al. Prevalence and clinical significance of acellular mucin in locally advanced rectal cancer patients showing pathologic complete response to preoperative chemoradiotherapy. *Am J Surg Pathol.* 2013;37(1):47–52.

Correspondence: Dr. Seok-Byung Lim sblim@amc.seoul.kr

Association between p16 expression and human papillomavirus in urinary bladder squamous cell carcinoma

Squamous cell carcinoma of the urinary bladder is unusual and of unknown etiology. There is a well-established association between human papillomavirus (HPV) infection and the development of cervical and head/neck squamous cell carcinomas. However, the role of HPV in the pathogenesis of squamous cell carcinoma of the urinary bladder is uncertain. The authors conducted a study to investigate the possible role of HPV in the development of squamous cell carcinoma of the urinary bladder and to determine if p16 expression could serve as a surrogate marker for HPV in this malignancy. In all, 42 cases of squamous cell carcinoma of the urinary bladder and 27 cases of urothelial carcinoma with squamous differentiation were investigated. HPV infection was analyzed by in situ hybridization at the DNA level and immunohistochemistry at the protein level. P16 protein expression was analyzed by immunohistochemistry. HPV DNA and protein were not detected in 42 cases of squamous cell carcinoma or 27 cases of urothelial carcinoma with squamous differentiation. P16 expression was detected in 13 cases (31 percent) of squamous cell carcinoma and nine cases (33 percent) of urothelial carcinoma with squamous differentiation. No correlation was found between p16 expression and the presence of HPV infection in squamous cell carcinoma of the bladder or urothelial carcinoma with squamous differentiation. The data suggest that HPV does not play a role in the development of squamous cell carcinoma of the urinary bladder or urothelial carcinoma with squamous differentiation. P16 expression should not be used as a surrogate marker for evidence of HPV infection in

squamous cell carcinoma of the urinary bladder or urothelial carcinoma with squamous differentiation as neither HPV DNA nor protein is detectable in these neoplasms.

Alexander RE, Hu Y, Kum JB, et al. P16 expression is not associated with human papillomavirus in urinary bladder squamous cell carcinoma. *Mod Pathol.* 2012;25(11):1526–1533.

Correspondence: Dr. L. Cheng at liang_cheng@yahoo.com

Immunohistochemical signature comprising PTEN, MYC, and Ki-67 and disease progression in prostate cancer

Loss of the tumor suppressor PTEN is common in prostate cancer and may have prognostic significance. The authors examined PTEN and additional protein markers in primary tumors from patients with high-risk, localized prostate cancer who received adjuvant docetaxel in the prospective multicenter trial TAX2501. Fifty-six of 77 patients enrolled in TAX2501 had primary prostatectomy specimens available for immunohistochemical analysis of PTEN, MYC, ERG, tumor protein p53 (p53), antigen Ki-67 (Ki-67), and phosphorylated forms of Akt, mammalian target of rapamycin, and S6 ribosomal protein. Protocol-defined progression included a prostate-specific antigen (PSA) level of 0.4 ng/mL or greater, radiologic/clinical recurrence, or death. Univariate and multivariable proportional hazards regression analyses were used to investigate the influence of PTEN status and other protein markers on progression-free survival. In this exploratory, post hoc analysis, PTEN protein loss was observed in 61 percent of patients and was associated with lower preoperative PSA levels, higher clinical stage, lower Ki-67 expression, and the presence of p53 and ERG. In univariate analysis, the factors associated with progression-free survival included Gleason sum, seminal vesicle invasion, PTEN status, MYC expression, and Ki-67 expression. In multivariable analysis, only three variables emerged as independent prognostic factors for progression-free survival: PTEN status (P=.035), MYC expression (P=.001), and Ki-67 expression (P<.001). A prognostic model was constructed that incorporated clinical covariates as well as information on PTEN, MYC, and Ki-67. The results indicated that PTEN status, MYC expression, and Ki-67 expression in primary tumor samples may predict progression-free survival more accurately than clinical factors alone in men with high-risk prostate cancer who receive adjuvant docetaxel after prostatectomy. If validated, these hypothesis-generating findings may have prognostic and therapeutic implications and may aid clinical trial design.

Antonarakis ES, Keizman D, Zhang Z, et al. An immunohistochemical signature comprising PTEN, MYC, and Ki67 predicts progression in prostate cancer patients receiving adjuvant docetaxel after prostatectomy. *Cancer.* 2012;118(24):6063–6071.

Correspondence: Dr. Mario A. Elseberger at eisenma@jhmi.edu

Preoperative BRAF(V600E) mutation screening: likelihood of altering initial surgery for indeterminate thyroid nodules

Preoperative B-type Raf kinase Val600Glu mutation, or BRAF(V600E), analysis has been proposed as a tool to guide initial surgery for indeterminate thyroid nodules. The authors conducted a study to determine if cytologic markers of malignancy are associated with the BRAF(V600E) mutation and if preoperative BRAF(V600E) testing would alter the initial management of patients with indeterminate nodules. Patients who underwent surgery for a thyroid nodule between 2003 and 2012 at a tertiary care center were prospectively enrolled. Stored nodule samples were retrospectively genotyped for the BRAF(V600E) mutation. The study examined BRAF(V600E) status, demographics, cytologic and histopathologic findings, and choice of initial surgery. Of the 960 patients enrolled, 310 (32 percent) had an indeterminate nodule. The BRAF(V600E) mutation was identified in 13 patients (four percent), 12 of whom had cytologic atypia or were Bethesda category V. Three percent of Bethesda category III or IV nodules that were malignant harbored the mutation compared with 42 percent of Bethesda category V malignancies. Nuclear grooves (P=.030), pseudoinclusions (P<.001), and oval nuclei (P=.022) were more common among BRAF(V600E) mutants.

The sensitivities of using BRAF testing alone, cytologic atypia/Bethesda category V classification, or both, were 15 percent, 73 percent, and 76 percent, respectively. Twelve of the 13 BRAF(V600E) mutants had total thyroidectomies initially due to worrisome cytologic features, and therefore the initial management of only one patient would have been altered if BRAF(V600E) testing had been performed preoperatively. The authors concluded that preoperative mutation screening for BRAF(V600E) does not meaningfully improve risk stratification and is unlikely to alter the initial management of patients with indeterminate nodules.

Kleiman DA, Sporn MJ, Beninato T, et al. Preoperative BRAF(V600E) mutation screening is unlikely to alter initial surgical treatment of patients with indeterminate thyroid nodules: A prospective case series of 960 patients. *Cancer.* 2013;119(8):1495–1502.

Correspondence: Dr. Thomas J. Fahoy III at tjfahoy@med.cornell.edu

Claudin expression in high-grade invasive ductal carcinoma of the breast

Claudin proteins are a major component of the tight junctions. Dysregulation of claudin protein expression has been described in a number of malignancies. Gene-expression profiling has stratified breast cancers into distinct molecular subtypes: luminal, HER2 positive (HER2+), and basal-like. A novel claudin-low molecular subtype recently has been described. The authors conducted a study in which they correlated the expression patterns of claudins with the molecular subtypes of breast cancer. On the basis of immunohistochemical expression, 226 grade 3 invasive ductal carcinomas were stratified into 65 luminal (estrogen receptor positive); 65 HER2+; 86 basal-like, including 14 metaplastic carcinomas (ER–, HER2–, CK5/6, and/or epidermal growth factor receptor positive); and 10 unclassified. Tissue microarrays were analyzed for the expression of claudins 1, 3, 4, 7, and 8 by immunohistochemistry and scored semiquantitatively. High levels of expression were detected in 17 percent of cases for claudin 1, 32 percent for claudin 3, 41 percent for claudin 4, 44 percent for claudin 7, and 40 percent for claudin 8. Luminal cancers exhibited increased claudins 7 and 8; basal-like tumors demonstrated increased expression of claudins 1 and 4. Low expression of all five claudins was detected in 30 of 226 cases (13 percent), and this group was designated “claudin-low.” The majority of the claudin-low subgroup were basal-like cancers (23 of 30, 77 percent). In contrast, only one of 30 (three percent) claudin-low tumors was of the luminal phenotype, and six of 30 cases (20 percent) were HER2+ ($P<.001$). Within the basal-like subgroup, 64 percent of the metaplastic and 19 percent of the nonmetaplastic tumors were claudin-low. The claudin-low group was strongly associated with disease recurrence ($P=.0093$). The authors concluded that claudin-low subtype is a frequent phenomenon in metaplastic and basal-like breast cancer and appears to be a strong predictor of disease recurrence.

Lu S, Singh K, Mangray S, et al. Claudin expression in high-grade invasive ductal carcinoma of the breast: correlation with the molecular subtype. *Mod Pathol.* 2013;26:485–495.

Correspondence: Dr. S. Lu at slu@lifespan.org

Interobserver agreement in assessing lung cancer: H&E diagnostic reproducibility for non-small cell lung carcinoma

Precise subtype diagnosis of non-small cell lung carcinoma is increasingly relevant based on the availability of subtype-specific therapies, such as bevacizumab and pemetrexed, and based on the subtype-specific prevalence of activating epidermal growth factor receptor mutations. The authors conducted a study to establish a baseline measure of interobserver reproducibility for non-small cell lung carcinoma diagnoses with hematoxylin-and-eosin for the current 2004 World Health Organization classification, estimate interobserver reproducibility for the therapeutically relevant squamous/nonsquamous subsets, and examine characteristics that improve interobserver reproducibility. For the study, they converted primary, resected lung cancer specimens to digital, or virtual, slides. Based on a single hematoxylin-and-eosin virtual slide, pathologists were asked to assign a diagnosis using the 2004 World Health Organization classification. Kappa statistics were calculated for each pathologist pair for each

slide and were summarized by classification scheme, pulmonary pathology expertise, diagnostic confidence, and neoplastic grade. Twelve pulmonary pathology experts and 12 community pathologists each independently diagnosed 48 to 96 single hematoxylin-and-eosin digital slides derived from 96 cases of non-small cell lung carcinoma resection. Overall agreement improved with simplification from the comprehensive 44 World Health Organization diagnoses (κ=0.25) to their 10 major header subtypes (κ=0.48), and improved again with simplification into the therapeutically relevant squamous/nonsquamous dichotomy (κ=0.55). Multivariate analysis showed that higher diagnostic agreement was associated with better differentiation and slide quality, higher diagnostic confidence, similar years of pathology experience, and pulmonary pathology expertise. The authors concluded that these data define the baseline diagnostic agreement for hematoxylin-and-eosin diagnosis of non-small cell lung carcinoma, allowing future studies to test for improved diagnostic agreement with reflex ancillary tests.

Grilley-Olson JE, Hayes DN, Moore DT, et al. Validation of interobserver agreement in lung cancer assessment: hematoxylin-eosin diagnostic reproducibility for non-small cell lung cancer: The 2004 World Health Organization classification and therapeutically relevant subsets. *Arch Pathol Lab Med.* 2013;137(1):32–40.

Correspondence: Dr. Neil Hayes at hayes@med.unc.edu and Dr. William K. Funkhouser at wfunkhou@unch.unc.edu