# Anatomic Pathology Selected Abstracts, 9/13

*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.*

## Quantification of the Ki67 proliferative index in neuroendocrine tumors of the gastroenteropancreatic system

Pathologic grading for prognostic stratification of neuroendocrine tumors is critical but presents a challenging interpretive dilemma. Tumor cell proliferative rate is an important factor in determining prognosis, and immunohistochemical analysis with Ki67 is becoming more widely used to quantify the proliferative rate. However, Ki67 assessment has limitations due to lack of uniformity and consistency in quantification. These limitations are accentuated in well-differentiated neuroendocrine tumors (NETs), as differences in the range of one percent to five percent can alter tumor grade, with potential implications for treatment. The authors performed a concordance study to assess different Ki67 quantification techniques, including digital image analysis, manual counting of more than 2,000 cells, and "eyeballed" estimates of labeling percentage by pathologists (n=18), including individuals experienced in evaluating Ki67 labeling as well as others who had little experience assessing Ki67 percentages. For the study, 45 Ki67 images were selected and analyzed using the three methods. On the basis of the recommendations of the World Health Organization for grading NETs, manual counting of 2,000 cells was used as the gold standard reference against which the other techniques were compared. Three images were presented twice, the second being inverted, to assess intraobserver consistency. Statistical analyses were performed to evaluate the concordance between methods, intraobserver and interobserver consistency, and correlation of NET grades on the basis of Ki67 scores by eyeballed estimates versus the gold standard. Agreement between scores was assessed by intraclass correlation (ICC). Digital image analysis and manual counting were highly concordant (ICC, 0.98). The ICC between digital image analysis and the mean eyeballed estimate of all observers was 0.88. However, there was discordance among individual observers on all cases quantified by eyeballed estimate (ICC, 0.13). The ICC for intraobserver consistency was 0.39±0.26. With Ki67 in the ranges of less than one percent, two percent to three percent, and more than 20 percent, the mean of Ki67 by eyeballed estimate was, respectively, 93±2 percent, 55±7 percent, and 55±15 percent correct against the gold standard. The › statistics for eyeballed estimate exhibited low agreement (›=0.24; 95 percent confidence interval, 0.23–0.25) for all WHO NET grades. Incorrect assessment by eyeballed estimate resulted in upgrading of all WHO G1 group tumors (n=14). In the WHO G2 group, downgrading of 41 percent of cases (n=11) occurred when Ki67 was less than five percent by digital image analysis and manual counting, and upgrading of 59 percent of cases (n=16) occurred when Ki67 was more than five percent. The authors concluded that digital image analysis and manual counting are the acceptable standards for Ki67 assessment. Given the inherent discordance in determining grade, use of an approximate eyeballed estimate of the Ki67-labeling index requires critical re-evaluation, especially for NETs with a labeling index straddling the cut points between grades. Consequently, determination of therapeutic strategies should be guided by an amalgamation of clinicopathologic characteristics, including but not limited to the Ki67 index.

Tang LH, Gonen M, Hedvat C, et al. Objective quantification of the Ki67 proliferative index in neuroendocrine tumors of the gastroenteropancreatic system: a comparison of digital image analysis with manual methods. *Am J Surg Pathol*. 2012;36(12):1761–1770.

Correspondence: Dr. Laura H. Tang at tangl@mskcc.org

## Clinicopathologic and immunohistochemical study of intrapulmonary SFTs

Solitary fibrous tumor is a ubiquitous neoplasm that arises most commonly from the pleura. SFT arising within lung parenchyma (intrapulmonary SFT) rarely has been reported and is therefore not well recognized. The authors presented a clinicopathologic and immunohistochemical study of 24 cases of primary intrapulmonary SFT. Patients

ranged in age from 44 to 83 years (mean, 58 years). None of the patients had evidence or history of a similar tumor elsewhere. Tumor size ranged from 2.3 to 22 cm (mean, 8.5 cm). On the basis of the degree of cytologic atypia, cellularity, mitotic activity, and areas of necrosis, the lesions were divided into low-grade, intermediate-grade, and high-grade histology. Twenty-one tumors showed the conventional features of SFT of low-grade histology (fewer than five mitoses per 10 high-power fields), with alternating bands of rope-like collagen flanked by a bland-appearing spindle cell proliferation. Hemangiopericytic, angiofibromatous, and a neural-like plexiform growth pattern were also observed. Five of 21 cases showed an "adenofibromatous" appearance imparted by entrapped normal air spaces at the advancing edges of the lesion. One intermediate-grade tumor showed overall increased cellularity with plump, pleomorphic nuclei—five to 10 mitoses per 10 high-power fields—and focal areas of classic SFT. Two cases showed high-grade features at initial presentation, with areas resembling a pleomorphic high-grade sarcoma admixed with foci of conventional, low-grade SFT. Immunohistochemical staining analyses performed in 13 cases showed positivity of the tumor cells for CD34, bcl-2, and CD99 in the majority of cases tested. Clinical followup was available for 18 patients, with long-term followup (more than five years) for six. Fourteen of 18 patients were alive and well without evidence of disease one month to 14 years after initial diagnosis. Three patients died of their tumors after four, five, and seven years; in two of them the initial tumor was of low-grade histology, but the recurrence/metastases showed a high-grade histology. The other fatal case showed a tumor with high-grade histology at initial diagnosis. One patient with intermediate-grade histology also had chest wall metastases at five years but was subsequently lost to followup. The authors concluded that the results of their study indicate that although tumors with overtly malignant histologic features can be expected to behave as high-grade sarcomas, tumors with bland-appearing morphologic features at presentation may also behave aggressively. Therefore, adequate excision with close clinical followup appears to be the most prudent course of action for managing primary intrapulmonary fibrous tumors.

Rao N, Colby TV, Falconieri G, et al. Intrapulmonary solitary fibrous tumors: clinicopathologic and immunohistochemical study of 24 cases. *Am J Surg Pathol.* 2013;37(2):155–166.

Correspondence: Dr. Nagarjun Rao at arao@mcw.edu

## Papillary mucinous metaplasia of the endometrium as a precursor of endometrial mucinous adenocarcinoma

Mucinous adenocarcinoma is an uncommon type of endometrial adenocarcinoma for which precursor lesions have yet to be clarified. During a review of noncancerous endometrial lesions in postmenopausal women, the authors found that mucinous endometrial glands showed variable degrees of epithelial changes that ranged from the formation of simple tubular glands to the formation of complex glands with papillary tufts. Some of the glands with papillary tufts were architecturally similar to low-grade mucinous adenocarcinomas. Based on histological similarities, the authors postulated that mucinous metaplasia could be a precursor lesion of mucinous adenocarcinoma. To explain the pathogenetic significance of endometrial mucinous metaplasia, the authors analyzed the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR), MK167, PTEN, β-catenin, p16 INK4A , TP53, and PAX2 in 21 endometrial mucinous metaplasias screened for KRAS (n=16) and PTEN (n=14) mutations. They also compared expression patterns between samples with simple mucinous glands, those with complex glands having intraglandular papillary tufts, and endometrioid adenocarcinomas. Compared with the surrounding flat mucinous epithelium and simple mucinous metaplasia, the intraglandular papillary tufts associated with papillary mucinous metaplasia were characterized by selectively decreased expression of PAX2 (P=.029) and PR (P<.001) and overexpression of p16 INK4A (P=.014). No significant differences were found between the two groups with regard to levels of expression of ER, PTEN, β-catenin, TP53, and MK167. In contrast with endometrioid adenocarcinomas, rates of MK167 proliferation were very low in both groups. Mutations in KRAS were identified in 89 percent of cases with papillary mucinous metaplasia, in contrast to 14 percent with simple mucinous metaplasia (P=.001). No PTEN mutations were observed in either of the two groups. The authors concluded that immunohistochemical and molecular genetic profiling suggest that papillary mucinous metaplasia is a possible precancerous lesion in a subset of endometrial carcinomas.

Yoo SH, Park BH, Choi J, et al. Papillary mucinous metaplasia of the endometrium as a possible precursor of endometrial mucinous adenocarcinoma. Mod Pathol. 2012;25:1496–1507.

Correspondence: Dr. K-R Kim at krkim@amc.seoul.kr

## Expression of miRNAs and PTEN in endometrial specimens

The authors investigated the relationship between frequently deregulated microRNAs and endometrial pathology to find the most dependable miRNA or combination of miRNAs to identify normal, hyperplastic, and malignant endometrial tissues. They also investigated the association between those miRNAs and phosphatase and tensin homolog (PTEN) status. They measured the expression of six miRNAs—miR-21, -182, -183, -200a, -200c, and -205—in 75 formalin-fixed, paraffin-embedded normal, hyperplastic, and malignant endometrial tissue blocks using Taqman-based real-time polymerase chain-reaction assays. PTEN loss of expression was assessed in the same endometrial tissues by immunohistochemistry. Expression of five miRNAs—miR-182, -183, -200a, -200c, and -205—was significantly higher in endometrial carcinoma than in complex atypical hyperplasia, simple hyperplasia, and normal endometrial tissue (P<.05, respectively). Considering the likelihood ratio and number of parameters, the composite panel of six miRNAs was the best marker, with a sensitivity of 91 percent and specificity of 94 percent for differentiating endometrial carcinoma from endometrial hyperplasia or normal endometrium. The individual miRNAs exhibited 64 percent to 77 percent sensitivity and 66 percent to 91 percent specificity. Interestingly, in distinguishing endometrial carcinoma from complex atypical hyperplasia, the composite panel of four miRNAs—miR-182, -183, -200a, and -200c—was the best marker, producing 95 percent sensitivity and 91 percent specificity. The percentage of PTEN loss was significantly higher in endometrial carcinoma compared with simple hyperplasia (68 percent versus 24 percent), and it was also higher in complex atypical hyperplasia compared with simple hyperplasia (71 percent versus 24 percent). Aberrant expression of miRNAs and loss of PTEN expression are common in endometrial hyperplasia and endometrial carcinoma. They might increase diagnostic reproducibility and improve discrimination, especially between complex atypical hyperplasia and endometrial carcinoma by miRNA expression profiles and between simple and complex hyperplasia through PTEN expression patterns. Those expression profiles of biomarkers also might be used to predict the potential for progression from endometrial hyperplasia to invasive endometrial carcinoma.

Lee H, Choi HJ, Kang CS, et al. Expression of miRNAs and PTEN in endometrial specimens ranging from histologically normal to hyperplasia and endometrial adenocarcinoma. Mod Pathol. 2012;25(11):1508–1515.

Correspondence: C. S. Park at charlie@catholic.ac.kr

## Molecular investigation of lymph nodes in colon cancer patients using OSNA

A new diagnostic system, called one-step nucleic acid amplification, detects cytokeratin 19 mRNA as a surrogate for lymph node metastases. The authors conducted a prospective investigation to compare the performance of one-step nucleic acid amplification (OSNA) with standard hematoxylin-and-eosin (H&E) analysis and intensive histopathology for detecting colon cancer lymph node metastases. In total, 313 lymph nodes from 22 consecutive patients with stages one, two, and three colon cancer were assessed. Half of each lymph node was analyzed initially by H&E followed by an intensive histologic workup (five levels of H&E and immunohistochemistry analyses, the gold standard for assessing the sensitivity and specificity of OSNA), and the other half was analyzed using OSNA. The authors found that OSNA was more sensitive for detecting small lymph node tumor infiltrates than H&E (11 results were OSNA positive and H&E negative). Compared with intensive histopathology, OSNA had 94.5 percent sensitivity, 97.6 percent specificity, and a concordance rate of 97.1 percent. OSNA resulted in an upstaging of two of 13 patients (15.3 percent) with lymph node-negative colon cancer after standard H&E examination. The authors concluded that OSNA appears to be a powerful and promising molecular tool for detecting lymph node metastases in patients with colon cancer. It performed similarly to intensive histopathologic investigations for detecting lymph node metastases and appeared to be superior to standard histology with H&E.

Furthermore, OSNA may lead to a potential upstaging of more than 15 percent of patients with colon cancer.

Góller U, Zettl A, Worni M, et al. Molecular investigation of lymph nodes in colon cancer patients using one-step nucleic acid amplification (OSNA): A new road to better staging? *Cancer.* 2012;118(24):6039–6045.

Correspondence: Dr. Markus Zuber at markus. zuber@spital.so.ch

## Thymidylate synthase expression and molecular alterations in adenosquamous carcinoma of the lung

Thymidylate synthase expression is higher in squamous cell carcinoma than in adenocarcinoma of the lung. It is thought that this is the reason for the poor efficacy of pemetrexed in squamous cell carcinoma. However, data are limited with regard to thymidylate synthase expression in adenosquamous carcinoma, a distinct subtype of lung cancer containing squamous and glandular differentiation. Furthermore, molecular alterations, such as epidermal growth factor receptor and Kirsten rat sarcoma 2 viral oncogene homolog mutations, which are seen in adenocarcinomas, are not well understood in mixed histology tumors, including adenosquamous carcinoma. The authors conducted a study to better characterize adenosquamous tumors of the lung. Using immunohistochemistry to evaluate thymidylate synthase protein levels, the authors found that expression of thymidylate synthase in these mixed tumors roughly paralleled that of squamous cell carcinoma, instead of falling in between squamous cell and adenocarcinoma. Of note, the expression of thymidylate synthase in adenosquamous samples was more closely correlated within the two components than would be expected by random chance alone. Furthermore, the authors noted a relatively high rate of epidermal growth factor receptor (11 percent) and Kirsten rat sarcoma 2 viral oncogene homolog (33 percent) mutations in these specimens, with the mutations showing convergence in the glandular and squamous components upon micro-dissection. The authors concluded that these results indicate that adenosquamous carcinomas are not simple mixtures of their two histological components but, rather, behave as distinct entities. This is important in further understanding their behavior. Given the similarity between thymidylate synthase expression in squamous cell and adenosquamous carcinoma, and that thymidylate synthase is the main target of pemetrexed, the authors extrapolated that pemetrexed may also have inferior clinical activity in adenosquamous carcinoma.

Shu C, Cheng H, Wang A, et al. Thymidylate synthase expression and molecular alterations in adenosquamous carcinoma of the lung. *Mod Pathol.* 2013;26:239–246.

Correspondence: Dr. A. C. Borczuk at ab748@columbia.edu

## Microdensitometry of osteopontin as a prognostic biomarker in colorectal carcinoma tissue microarrays

The authors conducted a study to explore the potential and limitations of “biomarker pathology” with quantitative immunohistochemistry on tissue microarrays, using osteopontin and colorectal carcinoma as a model system. Microdensitometry for quantitative evaluation of osteopontin immunohistochemistry (clone OP3N) on digital microphotographs using the public domain software ImageJ was observed to be straightforward and reliable. However, using colorectal carcinoma cell lines (n=11), the correlation between densitometric evaluations of Western blots and microdensitometry of immunocytochemistry of slide cultures was only moderate. A virtual resampling method to simulate tissue microarrays showed that, due to the heterogeneity of immunostaining, tumors were misclassified in nearly 20 percent of the arrays, even if four punches were used. Micro-densitometric evaluation of a tissue microarray made of a clinicopathologically well-characterized series of colorectal carcinomas with long-term followup (222 cases evaluable in the tissue microarray; Union Internationale Contre le Cancer [UICC] stages I–III/R0) showed a moderate survival advantage for patients with high osteopontin expression by microdensitometry. The authors concluded that these results challenge the basic assumption that microdensitometry is a precise technique for quantifying proteins detected by immunohistochemistry and delineate drawbacks when working with tissue microarrays in clinicopathological studies.

Prall F, Maletzki C, Linnebacher M. Microdensitometry of osteopontin as an immunohistochemical prognostic biomarker in colorectal carcinoma tissue microarrays: potential and limitations of the method in 'biomarker pathology'. *Histopathology.* 2012;61(5):823–832.

Correspondence: Dr. Friedrich Prall at friedrich.prall@med.unirostock.de

## Evaluation of pathological and molecular features in clinically aggressive dermatofibromas

Dermatofibroma, or cutaneous fibrous histiocytoma, represents a common benign mesenchymal tumor, and numerous morphological variants have been described. Some variants of dermatofibroma are characterized by an increased risk of local recurrences, and a few cases of metastasis have been reported. Unfortunately, aggressive behavior cannot be predicted reliably by morphology. The authors evaluated the value of array-comparative genomic hybridization in this setting. Seven cases of clinically aggressive dermatofibromas were identified, and pathological and molecular features were evaluated. The neoplasms occurred in four female and three male patients (mean age, 33 years; range, 2–65 years) and arose on the shoulder, buttock, temple, lateral neck, thigh, ankle, and cheek. The size of the neoplasms ranged from 1 cm to 9 cm (mean, 3 cm). An infiltration of the subcutis was seen in five cases. Two neoplasms were completely excised, and an incomplete or marginal excision was reported in the remaining cases. Local recurrences were seen in six cases (time to the first recurrence, eight months to nine years). Metastases were noted between three months and eight years after diagnosis in six patients. Two patients died of disease, and two patients were alive with disease. Histologically, the primary tumors showed features of cellular dermatofibroma (four cases), cellular/aneurysmal dermatofibroma (one case), atypical/cellular dermatofibroma (one case), and classical dermatofibroma (one case). Mitotic figures ranged from three to 25 per 10 high-power fields, and focal necrosis was present in five cases. Interestingly, malignant transformation from cellular dermatofibroma to an obvious spindle cell/pleomorphic sarcoma was seen in one primary and one recurrent neoplasm. Five neoplasms showed chromosomal aberrations by array-comparative genomic hybridization, suggesting that these changes may represent an additional diagnostic tool for identifying cases of dermatofibroma with metastatic potential.

Mentzel T, Wiesner T, Cerroni L, et al. Malignant dermatofibroma: clinicopathological, immunohistochemical, and molecular analysis of seven cases. *Mod Pathol.* 2013;26:256–267.

Correspondence: Dr. T. Mentzel at mentzel@dermpath.de

## Interobserver agreement in the reporting of colorectal polyp pathology by bowel cancer screening pathologists

The authors conducted a study to assess interobserver agreement in the reporting of colorectal polyps among histopathologists participating in the Welsh Bowel Cancer Screening (BCS) program. Twelve benign polyps representative of BCS cases were identified from pathology files and reported by 28 BCS histopathologists using proforma sheets. The level of agreement between the participants and a gold standard was determined using kappa statistics. A moderate level of agreement was achieved in the reporting of polyp type (›=0.45; 95 percent confidence interval [CI], 0.34–0.59), and adenomatous lesions were distinguished from nonadenomatous lesions in 96 percent of cases. Substantial agreement was obtained in distinguishing low- and high-grade dysplasias (›=0.67; 95 percent CI, 0.50–0.86), but there was only fair agreement in reporting excision margin status (›=0.24; 95 percent CI, 0.07–0.43), with frequent use of the "uncertain" category. Significant issues were noted in categorizing serrated lesions, recognizing focal high-grade dysplasia and epithelial misplacement, and diagnosing villous change in adenomas. The authors concluded that interobserver variability in some aspects of BCS pathologists' reporting of colorectal polyps is suboptimal, with a potential impact on patient management and efficient operation of the screening service.

Turner JK, Williams GT, Morgan M, et al. Interobserver agreement in the reporting of colorectal polyp pathology among bowel cancer screening pathologists in Wales. *Histopathology.* 2013;62(6):916–924.

Correspondence: S. Dolwani at sunil.dolwani@wales.nhs.uk