Anatomic Pathology Selected Abstracts, 7/14

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Assessing IHC biomarkers for basal-like breast cancer against a gene-expression profile gold standard

Gene-expression profiling of breast cancer delineates a particularly aggressive subtype referred to as basal-like. This subtype comprises approximately 15 percent of all breast cancers and afflicts younger women. It is refractory to endocrine and anti-HER2 therapies. Immunohistochemical surrogate definitions for basal-like breast cancer, such as the clinical ER/PR/HER2 triple-negative phenotype and models incorporating positive expression for CK5 (CK5/6) or EGFR, are heavily cited. However, many additional biomarkers for basal-like breast cancer have been described in the literature. A parallel comparison of 46 proposed immunohistochemical biomarkers of basal-like breast cancer was performed against a gene-expression profile gold standard on a tissue microarray containing 42 basal-like and 80 nonbasal-like breast cancer cases. Ki67 and PPH3 were the most sensitive biomarkers (both 92 percent) positively expressed in the basal-like subtype, whereas CK14, IMP3, and NGFR were the most specific (100 percent). Among the biomarkers surveyed, loss of INPP4B (a negative regulator of phosphatidylinositol signaling) was 61 percent sensitive and 99 percent specific with the highest odds ratio (OR) at 108, indicating the strongest association with basal-like breast cancer. Expression of nestin, a common marker of neural progenitor cells that is also associated with the triple-negative/basal-like phenotype and poor breast cancer prognosis, possessed the second highest OR at 29 among the 46 biomarkers surveyed, as well as 54 percent sensitivity and 96 percent specificity. As a positively expressed biomarker, nestin possesses technical advantages over INPP4B that makes it a more ideal biomarker for identifying basal-like breast cancer. The comprehensive immunohistochemical biomarker survey presented in this study is a necessary step for determining a surrogate immunopanel that best defines basal-like breast cancer in a practical and clinically accessible manner.

Won JR, Gao D, Chow C, et al. A survey of immunohistochemical biomarkers for basal-like breast cancer against a gene expression profile gold standard. *Mod Pathol.* 2013;26:1438–1450.

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Influence of sampling modality on predictive value of grading in adult soft tissue extremity sarcomas

Histologic grade is one of the best predictors of outcome in adult soft tissue sarcomas. Current grading systems were validated on resection specimens; however, there has been a trend toward the use of biopsies to diagnose these tumors. The authors conducted a study to determine whether the grade of an extremity soft tissue sarcoma determined on tissue obtained by core needle biopsy or incisional biopsy is predictive of metastasis- or disease-free survival and whether either sampling modality is superior. A total of 103 core needle biopsies and 107 incisional biopsies of nonmetastatic spindle cell sarcomas of the extremities were retrieved from the archives. All cases had a minimum two-year followup. Patient data and outcome and tumor characteristics were recorded. Tumors were reviewed and evaluated using the French Federation of Cancer Centers Sarcoma Group grading system. Kaplan-Meier survival curves were generated to correlate tumor grade with metastasis- and disease-free survival for both groups. The authors found that patient and tumor characteristics were similar between groups, except that more tumors were grade 3 and superficial in the incisional biopsy group. Grade determined on core needle biopsy was not predictive of metastasis-free survival ($P=0.59$) or disease-free survival ($P=0.50$). In contrast, grade determined on incisional biopsy was predictive of both metastasis-free survival ($P<0.001$) and disease-free

survival (P=0.001). The authors concluded that biopsy, particularly core needle biopsy, represents a convenient diagnostic tool, particularly in the context of neoadjuvant therapy. However, based on these results, incisional biopsy is recommended if grading is to be used to predict prognosis in spindle cell soft tissue sarcomas of the extremities.

Khoja H, Griffin A, Dickson B, et al. Sampling modality influences the predictive value of grading in adult soft tissue extremity sarcomas. *Arch Pathol Lab Med.* 2013;137:1774–1779.

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ARID1A expression and PI3K-Akt pathway alterations, TP53, and microsatellite instability in endometrial carcinogenesis

The switch/sucrose nonfermentable subunit ARID1A (AT-rich interactive domain 1A gene) has been postulated as a novel tumor suppressor of gynecologic cancer and a driver gene in endometrial carcinogenesis. However, its relationship to established molecular alterations in endometrioid endometrial cancer (EEC) is unknown. The authors analyzed the expression of ARID1A in 146 endometrial cancers (130 EECs and 16 non-EECs) in relation to alterations in the PI3K-Akt pathway (PTEN expression/KRAS/PIK3CA mutations), TP53 status (TP53 immunohistochemistry), and microsatellite instability. To discriminate between microsatellite instability due to somatic MLH1 promoter hypermethylation or germline mutations in one of the mismatch repair genes (Lynch syndrome), they included a Lynch syndrome set. This set included 21 cases with confirmed germline mutations and 15 cases that were suspected to have a germline mutation. Loss of ARID1A expression was exclusively found in 31 percent (40 of 130) of the EEC cases. No loss of expression of the other subunits of the switch/sucrose nonfermentable complex, SMARCD3 and SMARCB1, was detected. Alterations in the PI3K-Akt pathway were more frequent when ARID1A expression was lost. Loss of ARID1A and mutant-like TP53 expression was nearly mutually exclusive (P=0.0004). In contrast with the Lynch-associated tumors, a strong association between ARID1A loss and sporadic microsatellite instability was found. Only five cases (14 percent) of the Lynch syndrome set, compared with 24 cases (75 percent; P<0.0001) of the sporadic microsatellite-unstable tumors, showed loss of ARID1A. These observations suggest that ARID1A is a causative and not a target gene of microsatellite instability by playing a role in the epigenetic silencing of the MLH1 gene in endometrial cancer.
Bosse T, Ter Haar NT, Seeber LM, et al. Loss of ARID1A expression and its relationship with PI3K-Akt pathway alterations, TP53 and microsatellite instability in endometrial cancer. *Mod Pathol.* 2013;26:1525–1535.
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Discontinuous foci of cancer in a single core of prostatic biopsy

In addition to clinical data, prostatic biopsy reports help urologists outline a patient's treatment options. Discontinuous involvement of a core by multiple foci of cancer is not infrequent, but there is no consensus as to which method of quantification should be the standard. The authors used two distinct approaches to quantify the length of cancer foci in the prostatic biopsy and compared the results to radical prostatectomy (RP) parameters. All patients with matched prostatic biopsy and RP treated by the same medical team between 2006 and 2010 were consecutively included in the study. Tumor extent in the prostatic biopsy was estimated by multiple approaches, and the length was measured in millimeters. The subset of cases with discontinuous foci of cancer in a single core was initially reported by adding each foci and ignoring the benign intervening prostatic tissue, which was designated additive quantification (AQ). On slide review, these foci were reassessed as a single focus and measured by linear quantification (LQ). RPs were partially embedded according to the International Society of Urological Pathology recommendations, and the percentage of tumor was evaluated with graphic precision. Mean percentage of the tumor in RP (%RP) and in the prostatic biopsy were arbitrarily classified as limited (less than six percent) and nonlimited (six percent or more). Prostatic biopsy parameters were then correlated with %RP and margin status. All methods for quantifying the tumor in the prostatic biopsy obtained excellent correlation with %RP. Linear and additive quantification diverged in 14 of 38 patients, with a mean total length of cancer of 5.8 mm

more than the length obtained by linear quantification in the same population, accurately upgrading six of 14 cases to nonlimited. This subset (LQ>AQ) was more often seen in prostatic biopsy with significantly more positive cores (P=0.003) of predominantly Gleason score 7 and associated with positive surgical margins in RP (P=0.034) independent of %RP (21 percent versus 19 percent in the margin-negative cases). However, in the subset of prostatic biopsies in which the tumor infiltration was continuous (AQ=AL), positive margins were associated with tumor extent (31 percent versus six percent in margin-negative cases). Discontinuous foci of cancer in a single core were more often seen in prostatic biopsies sampling nonlimited disease, and this event was associated with positive surgical margins. Linear quantification of cancer improved the performance of the prostatic biopsies in predicting RP tumor extent relative to the traditional millimetric sum. The authors concluded that their findings support the idea that discontinuous foci may represent undersampling of a larger irregular nodule. However, this study was based on routine reports and did not directly access tumor biology.

Schultz L, Maluf CE, Da Silva RC, et al. Discontinuous foci of cancer in a single core of prostatic biopsy: when it occurs and performance of quantification methods in a private-practice setting. *Am J Surg Pathol.* 2013;37:1831–1836.

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Use of a 92-gene molecular cancer classifier to predict site of origin for neuroendocrine tumors

A diagnosis of neuroendocrine carcinoma is often morphologically straightforward; however, the tumor site of origin may remain elusive in a metastatic presentation. Neuroendocrine tumor subtyping has important implications for staging and patient management. In this study, the novel use and performance of a 92-gene molecular cancer classifier for determining the site of tumor origin are described in a series of 75 neuroendocrine tumors (44 metastatic and 31 primary: gastrointestinal [n=12], pulmonary [n=22], Merkel cell [n=10], pancreatic [n=10], pheochromocytoma [n=10], and medullary thyroid carcinoma [n=11]). Formalin-fixed, paraffin-embedded samples passing multicenter pathologist adjudication were blinded and tested by a 92-gene molecular assay that predicts tumor type/subtype based on relative quantitative PCR expression measurements for 87 tumor-related and five reference genes. The 92-gene assay demonstrated 99 percent accuracy (74 of 75; 95 percent confidence interval [CI], 0.93–0.99) for classifying neuroendocrine carcinomas and correctly subtyped the tumor site of origin in 95 percent of cases (71 of 75; 95 percent CI, 0.87–0.98). Analysis of gene-expression subsignatures within the 92-gene assay panel showed four genes with promising discriminatory value for tumor typing and 15 genes for tumor subtyping. The 92-gene classifier demonstrated excellent accuracy for classifying and determining the site of origin in tumors with neuroendocrine differentiation. The authors concluded that these results show promise for using this test to aid in classifying neuroendocrine tumors of indeterminate primary site, particularly in the metastatic setting.

Kerr SE, Schnabel CA, Sullivan PS, et al. A 92-gene cancer classifier predicts the site of origin for neuroendocrine tumors. *Mod Pathol.* 2014;27(1):44–54.

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Comparative IHC analysis of pulmonary and thymic neuroendocrine carcinomas

PAX8 is expressed in thymic epithelial neoplasms and a subset of neuroendocrine carcinomas of gastrointestinal origin but not in pulmonary neuroendocrine carcinomas. Thyroid transcription factor 1 (TTF-1) is known to be positive in pulmonary neuroendocrine carcinomas, but studies investigating its expression in thymic neuroendocrine carcinomas are lacking. The authors know of no comprehensive studies focusing on the comparative expression of PAX8 or TTF-1 in pulmonary and thymic neuroendocrine carcinoma. Consequently, a study was conducted in which 25 cases of low- and intermediate-grade neuroendocrine carcinomas of pulmonary

and thymic origin, respectively, were selected for immunohistochemical studies using antibodies directed against PAX8 and TTF-1. The percentage of positive tumor cells as well as the intensity of staining were evaluated and scored. Twenty-one of the pulmonary neuroendocrine carcinomas were classified as low-grade (typical carcinoid) and four as intermediate-grade (atypical carcinoid) tumors. The thymic tumors consisted of eight low-grade and 17 intermediate-grade neuroendocrine carcinomas. Only two (eight percent) of the pulmonary tumors showed nuclear expression of PAX8, while 19 (76 percent) expressed TTF-1. Of the thymic tumors, eight (32 percent) were positive for PAX8 and two (eight percent) showed TTF-1 positivity. Primary neuroendocrine carcinomas of the thymus are rare neoplasms that display a more aggressive clinical course than pulmonary neuroendocrine carcinomas, highlighting the importance of separating these tumors. No specific immunomarkers exist for distinguishing between neuroendocrine carcinomas of pulmonary and thymic origin. The differential expression of PAX8 and TTF-1 may prove useful in this context because a PAX8+/TTF-1− immunophenotype appears to be more common in thymic neuroendocrine carcinomas, whereas the reverse (PAX8−/TTF-1+) is true for most pulmonary neuroendocrine carcinomas.

Weissferdt A, Tang X, Wistuba II, et al. Comparative immunohistochemical analysis of pulmonary and thymic neuroendocrine carcinomas using PAX8 and TTF-1. *Mod Pathol.* 2013;26:1554–1560.

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