Anatomic Pathology Selected Abstracts, 8/14

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GATA3: a multispecific but potentially useful marker in surgical pathology

The transcription factor GATA3 is important for differentiating breast epithelia, urothelia, and subsets of T lymphocytes. It has been suggested that it may be useful in evaluating carcinomas of mammary or urothelial origin or metastatic carcinomas, but its distribution in normal and neoplastic tissues is incompletely mapped. The authors conducted a study in which they examined normal developing and adult tissues and 2,040 epithelial and 460 mesenchymal or neuroectodermal neoplasms for GATA3 expression to explore its diagnostic value in surgical pathology. They used monoclonal antibody (clone L50-823) and Leica Bond automated immunohistochemistry. GATA3 was expressed in trophoblast, fetal and adult epidermis, adult mammary and some salivary gland and sweat gland ductal epithelia, urothelia, distal nephron in developing and adult tissues, some prostatic basal cells, and subsets of T lymphocytes. It was expressed more strongly in fetal than in adult mesothelia and was absent in respiratory and gastrointestinal epithelia. In epithelial neoplasms, GATA3 was expressed in more than 90 percent of primary and metastatic ductal and lobular carcinomas of the breast, urothelial and cutaneous basal cell carcinomas, and trophoblastic and endodermal sinus tumors. In metastatic breast carcinomas, it was more sensitive than gross cystic disease fluid protein. Among squamous cell carcinomas, the expression was highest in the skin (81 percent) and lower in cervical (33 percent), laryngeal (16 percent), and pulmonary tumors (12 percent). Common positivity was found in skin adnexal tumors (100 percent), mesothelioma (58 percent), salivary gland (43 percent), and pancreatic (37 percent) ductal carcinomas, whereas frequency of expression in adenocarcinomas of lung, stomach, colon, endometrium, ovary, and prostate was less than 10 percent. Chromophobe renal cell carcinoma was a unique renal tumor with frequent positivity (51 percent), whereas oncocytomas were positive in 17 percent of cases but other types only rarely. Among mesenchymal and neuroectodermal tumors, paragangliomas were usually positive, which sets these tumors apart from epithelial neuroendocrine tumors. Mesenchymal tumors were only sporadically positive, except epithelia of biphasic synovial sarcomas. The authors concluded that GATA3 is a useful marker for characterizing not only mammary and urothelial carcinomas but also renal and germ cell tumors, mesotheliomas, and paragangliomas. The multiple specificities of GATA3 should be taken into account when using this marker to detect metastatic mammary or urothelial carcinomas.

Miettinen M, McCue PA, Sarlomo-Rikala M, et al. GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. *Am J Surg Pathol*. 2014;38:13–22.

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Chromophobe hepatocellular carcinoma with abrupt anaplasia

Hepatocellular carcinomas exhibit heterogeneous morphologies by routine light microscopy. Although some morphologies represent insignificant variations in growth patterns, others may represent unrecognized subtypes of hepatocellular carcinoma. Identification of these subtypes could lead to separating hepatocellular carcinomas into discrete groups with unique underlying genetic changes, prognoses, or therapeutic responses. To identify potential subtypes, two pathologists conducted a study in which they independently screened a cohort of 219 unselected hepatocellular carcinoma resection specimens and divided the cases into potential subtypes. One of these promising candidate subtypes was further evaluated using histological and molecular techniques. This subtype was characterized by a unique and consistent set of histological features: smooth chromophobic cytoplasm; abrupt focal nuclear anaplasia (small clusters of tumor cells with marked nuclear anaplasia in a background of tumor cells

with bland nuclear cytology); and scattered microscopic pseudocysts, which the authors designate as chromophobe hepatocellular carcinoma with abrupt anaplasia. Thirteen cases were identified (six percent of all hepatocellular carcinomas), comprising six men and seven women who were an average age of 61 years. Six cases occurred in cirrhotic livers. Serum alfa-fetoprotein was elevated in six out of 10 (60 percent) cases. A variety of underlying liver diseases were noted, but cases were enrichment for chronic hepatitis B (P=0.006). Interestingly, at the molecular level, this variant was strongly associated with the alternative lengthening of telomere (ALT) phenotype by telomere FISH. ALT is a telomerase-independent mechanism of telomere maintenance and is found in approximately eight percent of unselected hepatocellular carcinomas. In contrast, 11 of 12 (92 percent) of the cases of chromophobe hepatocellular carcinoma with abrupt anaplasia were ALT-positive. The authors propose that chromophobe hepatocellular carcinoma with abrupt anaplasia represents a new subtype of hepatocellular carcinoma with unique morphological and molecular features.

Wood LD, Heaphy CM, Daniel HD, et al. Chromophobe hepatocellular carcinoma with abrupt anaplasia: a proposal for a new subtype of hepatocellular carcinoma with unique morphological and molecular features. *Mod Pathol*. 2013;26:1586–1593.

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Standard HER2 testing of endometrial serous carcinoma—experience at a large academic center

HER2 overexpression or amplification, or both, has been reported in endometrial serous carcinoma, suggesting that HER2 may be a promising therapeutic target. However, considerable variation exists in the reported rates of HER2 overexpression and amplification, likely, at least in part, resulting from variability in the testing methods, interpretation, and scoring criteria used. Unlike in breast and gastric cancer, there are no established guidelines for HER2 testing in endometrial carcinoma. A study was conducted in which 108 endometrial carcinoma cases—85 pure serous carcinomas and 23 mixed endometrial carcinomas with serous component—were identified over a four-year period at a large academic center. All H&E and HER2 immunohistochemical slides were reviewed, and HER2 FISH results (available on 52 cases) were retrieved from pathology reports. HER2 immunohistochemical scores were assigned according to FDA criteria and breast ASCO/CAP scoring criteria. Clinical information was retrieved from the patients' medical records. Thirty-eight (35 percent) cases showed HER2 overexpression or gene amplification, or both, 20 (53 percent) of which had significant heterogeneity of protein expression by immunohistochemistry. Lack of apical membrane staining resulting in a lateral/basolateral staining pattern was observed in the majority of HER2-positive tumors. Five (13 percent) of the HER2-positive cases demonstrated discrepant immunohistochemical scores when using the FDA versus ASCO/CAP scoring system. The overall concordance rate between HER2 immunohistochemistry and FISH was 75 percent (39 of 52) when using the FDA criteria, compared with 81 percent (42 of 52) by the ASCO/CAP scoring system. The authors concluded that in this large comprehensive study, 35 percent of endometrial serous carcinomas harbor HER2 protein overexpression or gene amplification, or both, over half of which demonstrate significant heterogeneity of protein expression. The breast ASCO/CAP scoring criteria provide the highest concordance between immunohistochemistry and FISH. Assessment of HER2 immunohistochemistry on multiple tumor sections or sections with large tumor areas is recommended due to the significant heterogeneity of HER2 protein expression.

Buza N, English DP, Santin AD, et al. Toward standard HER2 testing of endometrial serous carcinoma: 4-year experience at a large academic center and recommendations for clinical practice. *Mod Pathol*. 2013;12:1605–1612.

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Discontinuous foci of cancer in a single core of prostatic biopsy

Prostate biopsy reports orient urologists in outlining a patient's treatment options. Discontinuous involvement of a core by multiple foci of cancer is not infrequent; however, 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 prostate biopsy and compared the results to prostatectomy parameters. All patients with matched prostate biopsy and prostatectomy treated by the same medical team between 2006 and 2010 were included consecutively in the study. Tumor extent in the prostate 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 as additive quantification (AQ). On slide review, these foci were reassessed as a single focus and measured by linear quantification (LQ). Prostatectomies 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 prostatectomy (%RP) and in the prostate biopsy were arbitrarily classified as limited (less than six percent) and nonlimited (six percent or more). Prostate biopsy parameters were then correlated with %RP and margin status. All methods of quantification of the tumor in the prostate 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 LQ in the same population, accurately upgrading six of 14 cases to nonlimited. This subset (LQ>AQ) was more often seen in prostate biopsy with significantly more positive cores (P=0.003) of predominantly Gleason score 7 and associated with positive surgical margins in prostatectomy (P=0.034) independent of %RP (21 versus 19 percent in the margin-negative cases). However, in the subset of prostate biopsy in which the tumor infiltration was continuous (AQ=AL), positive margins were associated with tumor extent (31 versus six percent in margin-negative cases). Discontinuous foci of cancer in a single core were most often seen in prostate biopsy sampling nonlimited disease, and this event was associated with positive surgical margins. Linear quantification of cancer improved the performance of the prostate biopsy in predicting prostatectomy 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 is based on routine reports and does 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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Tumor budding in rectal cancer biopsies before neoadjuvant therapy

The authors conducted a study to correlate intra-tumoral budding in pretreatment rectal cancer biopsies with pathological response to neoadjuvant chemoradiotherapy and long-term outcome. Data from a prospectively maintained database were acquired from patients with locally advanced rectal cancer who underwent neoadjuvant chemoradiotherapy. Pretreatment rectal biopsies were retrospectively reviewed for evidence of intra-tumoral budding. Multivariate logistic regression was used to identify factors contributing to cancer-specific death, expressed as hazard ratios with 95 percent confidence intervals. Of 185 patients with locally advanced rectal cancer, 89 met the eligibility criteria, of whom 18 (20 percent) exhibited budding in a pretreatment tumor biopsy. Intra-tumoral budding predicted a poor pathological response to neoadjuvant chemoradiotherapy (higher ypT stage, P=0.032; lymph node involvement, P=0.018; lymphovascular invasion, P=0.004; and residual poorly differentiated tumors, P=0.005). No patient with intra-tumoral budding exhibited a tumor regression grade 1 or complete pathological response, providing 100 percent specificity and positive predictive value for nonresponse to neoadjuvant chemoradiotherapy. Intra-tumoral budding was associated with a lower disease-free five-year survival rate (33 versus 78 percent; P<0.001), cancer-specific five-year survival rate (61 versus 87 percent; P=0.021), and predicted cancer-specific death (hazard ratio, 3.51; 95 percent confidence interval, 1.03–11.93; P=0.040). The authors concluded that intra-tumoral budding at diagnosis of rectal cancer identifies those who will respond poorly to neoadjuvant chemoradiotherapy and those with a poor prognosis.

Rogers AC, Gibbons D, Hanly AM, et al. Prognostic significance of tumor budding in rectal cancer biopsies before neoadjuvant therapy. *Mod Pathol*. 2014;27:156–162.

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Analysis of diagnostic criteria for very well-differentiated gastric carcinoma of intestinal type

Very well-differentiated gastric adenocarcinoma of intestinal type is a rare variant of gastric cancer characterized by low-grade nuclear atypia. Its diagnostic criteria and clinical behavior are not fully established. The authors presented a detailed histologic, immunohistochemical, and clinical analysis of 21 cases. Nuclear atypia was mild in all of them. Characteristic architectural features of this gastric adenocarcinoma variant were pit and glandular anastomosis, spiky glands, distended glands, discohesive cells, abortive glands, and glandular outgrowth. At least three of these features were present in all cases. Retrospective review of preoperative biopsies in 18 patients revealed that half the biopsies were originally reported as negative or indeterminate for malignancy. On the basis of immunohistochemical stains for intestinal (MUC2, CD10, and CDX-2) and gastric (MUC5AC and MUC6) markers, 11 (52 percent) cases had an intestinal immunophenotype and 10 (48 percent) cases had a mixed immunophenotype. Foci of discohesive neoplastic cells, indicating dedifferentiation toward a poorly cohesive carcinoma, were observed exclusively in neoplasms of mixed immunophenotype (n=5). All but one patient with follow-up were alive without disease at a mean of 19 months (range, 1–60 months). One individual with a pT4 tumor with associated poorly cohesive carcinoma died of disease. The authors concluded that very well-differentiated gastric adenocarcinomas are diagnostically challenging. Architectural features are critical to making the diagnosis. Cases with pure intestinal immunophenotype have not been associated with transformation into poorly cohesive carcinoma and appear to behave as biologically low grade. Those with mixed immunophenotype appear more likely to dedifferentiate and behave more aggressively.

Ushiku T, Arnason T, Ban S, et al. Very well-differentiated gastric carcinoma of intestinal type: analysis of diagnostic criteria. *Mod Pathol*. 2013;26:1620–1631.

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A study of cystic hypersecretory carcinoma of the breast

Cystic hypersecretory carcinoma is an uncommon variant of ductal carcinoma in situ characterized by, among other features, the presence of luminal secretion resembling thyroidal colloid. It is thought to behave in an indolent manner but has the potential to give rise to invasive carcinoma, which is often poorly differentiated. The authors studied the immunohistochemical, clinical, and morphologic features of 10 cases of cystic hypersecretory carcinoma (CHC). All patients were women who were an average of 62.8 years old (range, 47–79 years). The clinical/radiographic presentation was a mass (five of 10), calcifications (three of 10), bloody nipple discharge (one of 10), and unknown (one of 10). The microscopic size of CHC ranged from 0.2 to 2.7 cm (mean, 0.9 cm). Micropapillary growth was present in all cases. Nuclear grade was intermediate (five of 10) or high (five of 10). One case also showed microinvasive carcinoma. All cases arose in a background of cystic hypersecretory hyperplasia or cystic hypersecretory hyperplasia with atypia, or both. CHC was ER+ in eight of 10 cases (ER+/PR+, four of 10; ER+/PR−, four of 10). Two cases were ER−/PR−, including the case with microinvasive carcinoma. All cases were HER2−. Androgen receptor was expressed in three of 10 cases. The myoepithelial stains p63, smooth muscle myosin, and CK5 showed circumferential staining in nine of 10 cases, whereas one case was negative for p63, smooth muscle myosin, and CK5 in CHC and adjacent cystic hypersecretory hyperplasia. The basal-like carcinoma markers EGFR, CK14, and CK5 were negative in all cases, with the exception of one case that was positive for EGFR. Four patients with follow-up information showed no evidence of disease (mean, 5.5 years). CHC is a distinct variant of ductal carcinoma in situ that arises in a background of cystic hypersecretory hyperplasia and is characterized by micropapillary growth, intermediate- to high-grade nuclei, and luminal colloid-like secretion. It is usually ER+ and HER2−. Negative or discontinuous reactivity with myoepithelial markers may be seen, despite its in situ nature. CHC usually behaves in a nonaggressive manner, as was seen in the authors' patients, all of whom were free from disease at last follow-up.

D'Alfonso TM, Ginter PS, Liu YF, et al. Cystic hypersecretory (in situ) carcinoma of the breast: a clinicopathologic and immunohistochemical characterization of 10 cases with clinical follow-up. *Am J Surg Pathol*. 2014;38:45–53.

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OCT4 immunohistochemistry to evaluate retroperitoneal lymph node dissections

The authors investigated the role of OCT4 immunohistochemical staining in detecting germ cell tumor lymph node metastases. Retroperitoneal lymph node dissection is important for staging and treating testicular germ cell tumors, and OCT4 is sensitive and specific for pluripotent testicular germ cell tumors. However, micrometastases, particularly from seminoma, can be difficult to detect. The authors examined 262 lymph nodes in 45 retroperitoneal lymph node dissection specimens from germ cell tumor patients. They categorized specimens as postchemotherapy and untreated retroperitoneal lymph node dissection with or without clinical suspicion, based on lymphadenopathy or elevated serum germ cell tumor markers. Sections were stained with anti-OCT4 antibody. Twenty-one additional positive lymph nodes in 12 cases were found to harbor scattered seminoma cells, singly and in small clusters, from 256 previously considered benign in untreated retroperitoneal lymph node dissection with clinical suspicion (13 percent increase), postchemotherapy retroperitoneal lymph node dissection (seven percent), and untreated retroperitoneal lymph node dissection without suspicion (four percent). No patient with an entirely negative dissection specimen was reclassified as positive. OCT4 immunohistochemistry detected scattered seminoma cells and small clusters of seminoma cells in lymph nodes previously considered to be benign, for an overall increase of eight percent and greatest in the setting of untreated retroperitoneal lymph node dissection with clinical suspicion. Immunohistochemistry did not convert any entirely negative specimen to positive. The authors concluded that additional studies will be useful to determine whether the small volume of disease detected by immunohistochemistry has the same impact as lymph node metastases detected routinely.

Idrees MT, Williamson SR, Kieffer TW, et al. The role of OCT4 immunohistochemistry in evaluation of retroperitoneal lymph node dissections: a pilot study. *Mod Pathol*. 2013;26:1613–1619.

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EGFR alterations and EML4-ALK rearrangement in primary adenocarcinoma of the urinary bladder

The identification of mutations in epidermal growth factor receptor and translocations involving anaplastic lymphoma kinase in lung adenocarcinoma has drastically changed understanding of the disease and led to the development of targeted therapies. Adenocarcinoma of the urinary bladder is rare and poorly understood at the molecular level. The authors conducted a study to determine whether epidermal growth factor receptor (EGFR) mutations, increases in EGFR copy number, or anaplastic lymphoma kinase (ALK) translocations are present in these tumors. They analyzed 28 cases of primary bladder adenocarcinoma. For EGFR mutational analysis, they analyzed PCR-amplified products on the Q24 pyrosequencer with Qiagen EGFR Pyro kits. All cases were analyzed via FISH using Vysis ALK Break Apart FISH probes for detecting ALK chromosomal translocation and Vysis dual-color probes to assess for increased gene copy number of EGFR. None of the 28 cases examined showed mutational events in EGFR or ALK rearrangements. EGFR polysomy was seen in 10 of 28 (36 percent) cases. No correlation with EGFR polysomy was seen in the tumors with respect to age, histologic subtypes, pathologic stage, or lymph node metastasis. The authors concluded that EGFR mutations and ALK rearrangements do not appear to be involved in the development of primary adenocarcinoma of the urinary bladder. However, a subgroup of cases (36 percent) demonstrated increased gene copy number of EGFR by FISH.

Alexander RE, Montironi R, Lopez-Beltran A, et al. EGFR alterations and EML4-ALK rearrangement in primary adenocarcinoma of the urinary bladder. *Mod Pathol*. 2014;27:107–112.

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