A Diagnostic Immunohistochemistry Update

Subspecialties in Anatomic Pathology

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I am grateful for the opportunity to organize this Diagnostic Immunohistochemistry (IHC) Update Special Section, mainly with contributions from my colleagues at Geisinger Health System (Danville, Pennsylvania). It consists of 8 review articles attempting to cover most subspecialties in anatomic pathology in the limited space available. In contrast to the 2 IHC Update Special Issues published in December 2014 and January 2015 that focused on both a comprehensive review and updates in diagnostic IHC, most of the review articles in this special section focus on recent advances, including new diagnostic markers, the most effective IHC panels, and markers for new entities, with only a brief review of the literature.

This special section begins with an article discussing one of the most frequent and important applications of diagnostic IHC, that is, to assist in working up an undifferentiated neoplasm/tumor of uncertain primary site on fine-needle aspiration (FNA) and small tissue biopsy specimens. The first article concisely reviews some diagnostic strategies and includes a comprehensive working algorithm. Some of these important diagnostic strategies can be briefly summarized as (1) prepare 2 cell blocks/tissue blocks if possible; (2) categorize an undifferentiated tumor based on cytologic/morphologic features into epithelioid, spindle, small round cell, etc; (3) correlate with previous molecular testing; (4) start with a small panel of broad-screening IHC markers followed by a second “differentiation-specific” panel; (4) be aware of newly described IHC markers; and (5) know when “enough is enough” and maximally preserve the precious tumor tissue for prognostic and therapeutic IHC markers or molecular testing.

The second article describes new IHC markers to address some of the frequently encountered diagnostic issues in the liver, gastrointestinal, and pancreaticobiliary tract, such as (1) the effective diagnostic panel, including von Hippel-Lindau protein (pVHL), mammary serine protease inhibitor (maspin), S100 calcium-binding protein P (S100P), and insulin-like growth factor 2 mRNA-binding proteins (IMP3 or KOC), in diagnosing pancreatic ductal adenocarcinoma; (2) B-cell lymphoma/leukemia 10 (BCL10) and carboxypeptidase A1 (CPA1) as the most sensitive and specific markers for confirming a diagnosis of acinar cell carcinoma of the pancreas; (3) a panel consisting of soluble suppression of tumorogenesis-2 (SST2), α-thalassemia/mental retardation X-linked (ATRX), death domain-associated protein (DAXX), p53, and retinoblastoma 1 (RB1) to be helpful in differentiating pancreatic neuroendocrine tumor, grade 3 (PanNET G3) from pancreatic neuroendocrine carcinoma (PanNEC), with most PanNECs being negative for SST2, with a mutation pattern of p53 and loss of RB1; (4) markers to confirm a diagnosis of intrahepatic cholangiocarcinoma, such as C-reactive protein (CRP), hepatocyte nuclear factor-1β (HNF-1β), and albumin (by RNA in situ hybridization [ISH]); and (5) markers for subtyping hepatic adenoam.

The third review article focuses on updates in IHC markers for salivary glands. Newly described entities are included based on the upcoming the 5th edition of the WHO Classification of Head and Neck Tumours: Salivary Glands. Emerging diagnostic IHC markers have played a crucial role in the diagnosis and differential diagnosis of salivary gland tumors, especially in FNA/small tissue biopsy samples. For surgical pathologists and cytopathologists, it is imperative to be aware of these newly described IHC markers, such as pleomorphic adenoma gene 1 (PLAG1) for pleomorphic adenoma, nuclear β-catenin staining for basal cell adenoma, nuclear receptor subfamily 4 group A member 3 (NR4A3) for acinic cell carcinoma, activating neurotrophic tyrosine receptor kinase fusions (pan-TRK) for secretory carcinoma, MYB (by RNA ISH) for adenoid cystic carcinoma, and HRAS Q61 for de novo epithelial-myoepithelial carcinoma. Diagnostic sensitivity, specificity, and potential pitfalls of each marker are delineated.

Part II of this special section begins with a review of frequently used and newly described IHC markers (such as NK3 homeobox 1 [NKX3.1], NK6 homeobox 1 [NKX6-1], S- [2-succinyl] cysteine [2SC], zinc finger and BTB domain-containing protein 16 [ZBTB16], forkhead box L2 [FOXL2], and insulin-like 3 [INSL3]) in the diagnosis and differential diagnosis of both benign and neoplastic lesions in genitourinary organs, including kidney, bladder, prostate, and testis. Updates from the 2022 WHO Classification of Urinary and Male Genital Tumours are reflected. IHC markers for newly classified renal epithelial neoplasms and their differential diagnosis are listed. The article focuses on the most effective IHC panels for a difficult differential diagnosis, including (1) variants of urothelial carcinoma and their mimickers; (2)
differential of high-grade prostatic adenocarcinoma with urothelial carcinoma; and (5) distinction between germ cell tumors and sex cord stromal tumors. Frequently encountered pitfalls in the daily interpretation of IHC are also emphasized.

Many entities in breast and gynecologic pathology would benefit from evaluation with various IHC stains. Perhaps TRPS1 is one of the most important novel IHC markers recently described because of its superior diagnostic sensitivity and specificity in identifying breast primary and expression in most triple-negative breast carcinomas. IHC is also used to distinguish new entities, such as mesonephric-like endometrial adenocarcinoma (MEC), from their mimics. Like endometrial adenocarcinoma, MECs express paired box gene 8 (PAX8). However, they also express thyroid transcription factor-1 (TTF-1) and/or GATA3, but they do not express estrogen receptors (ERs) or progesterone receptors (PRs). TTF-1 and GATA3 have also been described as having inverse staining patterns in MEC, with TTF-1 expression and GATA3 negativity the most common finding. Updated guidelines for recommended ancillary studies, such as mismatch repair proteins, p53, and human epidermal growth factor receptor 2 (HER2) studies in endometrium, as well as ER, PR, and HER2 in breast, are discussed.

In the review article on the utility of IHC in the diagnosis of pleuropulmonary and mediastinal tumors, which begins part III of our special section, the author emphasizes the important role of IHC in assisting in the diagnosis and classification of these tumors, particularly in small biopsy/cytologic specimens. Updates on the diagnosis and classification of thoracic tumors are based on the 2021 WHO Classification of Thoracic Tumours. Topics include (1) the differentiation between adenocarcinoma and squamous cell carcinoma; (2) IHC for neuroendocrine neoplasms; (3) the utility of IHC on undifferentiated carcinoma and uncommon pulmonary cancers; (4) the distinction between primary lung and metastatic carcinomas; (5) the diagnosis of mesotheliomas and their differentiation from reactive mesothelial proliferations and from metastasis; and (6) IHC application in thymic tumors. In addition, the author also addresses the pros and cons in selection of the antibody panel, antibody clones, and pitfalls in interpretation of the staining results.

Many molecular alteration–related IHC markers have recently been discovered in the field of soft tissue, bone, and skin (particularly for melanoma). In the next review article, the authors selected a group of recently described IHC markers that have proven to be very useful, and they provide an in-depth discussion of each marker’s diagnostic sensitivity, specificity, and potential pitfalls, for example, (1) BRCA1–associated protein 1 (BAP1) for BAP1-inactivated melanocytic tumors; (2) preferentially expressed antigen in melanoma (PRAME) for melanocytic lesions; (3) SS18–SSX for synovial sarcoma; (4) DDIT3 for myoid liposarcoma; (5) histone H3 lysine 27 trimethylation (H3K27Me3) for malignant peripheral nerve sheath tumor; and (6) G34W for giant cell tumor of bone.

The last review article on updates in IHC for hematopoietic and lymphoid neoplasms (1) discusses the use of new immunohistochemical markers for B-cell lymphomas since the 2014 review published by the Archives of Pathology & Laboratory Medicine; (2) reviews additional new immunohistochemical stains in the diagnostic evaluation of B-cell lymphoma after the 2014 review, such as cyclin D3 for splenic diffuse red pulp small B-cell lymphomas and myeloid and lymphocyte protein (MAL, by RNA ISH) for primary mediastinal large B-cell lymphoma; (3) reviews the new IHC markers in the diagnostic evaluation of T-cell lymphoma, such as overexpression of CD28 in adult T-cell leukemia/lymphoma showing a significantly poorer overall survival, and overexpression of LIM domain–only 2 (LMO2) in most T-cell lymphoblastic lymphomas but not in immature terminal deoxynucleotide transferase–positive T cells in the thymus or indolent T-lymphoblastic proliferations; (4) updates new immunohistochemical stains in the diagnostic evaluation of myeloid neoplasms, such as IRF8 expression in the identification of monoblasts and expression of glucose transporter 1 (GLUT1) in both benign and neoplastic erythroid population in bone marrow biopsies; and (5) reviews IHC markers, such as FMS-like tyrosine kinase (FLT3), inhibitor of DNA binding 1 (ID1), and isocitrate dehydrogenase 2 (IDH2), to be used as an early surrogate for molecular diagnosis of acute myeloid leukemias.

Finally, applications of RNA ISH technology in anatomic pathology have gained in popularity in recent years. Because of the limited space, this special section does not cover this emerging novel technology. However, it is worthwhile to briefly summarize the applications of this new technology here. For additional information, frequently asked questions, and references, one can refer to the recently published book chapter (Advanced Cell Diagnostics, Hayward, California), have dramatically improved the performance of this approach and led to the development of RNA ISH assays for a variety of applications across anatomic pathology. RNA ISH assays are highly sensitive and specific, highly reproducible, and can be run on an automated IHC platform like traditional IHC. These applications can be briefly summarized as follows: (1) detection of high-risk types of human papillomavirus (HPV) in head and neck squamous cell carcinomas, an application with both high diagnostic sensitivity and specificity when compared to other detection techniques, such as DNA ISH and p16 immunohistochemical stain; (2) detection of immunoglobulin lambda (Igλ) chain gene fusion (for example, ALK, NTRK1, NTRK3, and pancreatic-duodenal homeobox gene 1 [PDX1]) in gastric adenocarcinoma; (3) detection of oncoprotein fusion (e.g., ALK, NTRK1, NTRK3, and pancreatic-duodenal homeobox gene 1 [PDX1]) in gastric adenocarcinoma; (4) detection of DNA-repair gene mutations, such as TP53 and ATM, in lung adenocarcinomas; (5) detection of gene fusions, such as those involving the PD1 and PD2 genes, potentially providing an alternative approach to fluorescence ISH and other technologies.

In summary, many recently described entity-specific IHC markers are related to molecular alterations, such as translocation/gene fusion (for example, ALK, NTRK1, NTRK3, SS18–SSX, and pan-TRK), point mutation (BRAF V600E, H3K27Me3, and G34W), deletion (SMARCA4, RB, BAP1, ATRX, and DAXX), and amplification (HER2, cyclin-dependent kinase 4 [CDK4], and mouse double minute 2 [MDM2]). With the advances of molecular pathology, this trend will continue and many more molecular alteration–related IHC markers will be...
discovered. With more specific IHC markers available, a more effective and smaller IHC panel can be applied when working up a challenging case; and importantly, precious tumor specimens can be maximally preserved for relevant therapeutic and prognostic molecular tests. Additionally, RNA ISH will continue proving its clinical utilities and gaining popularity, and potentially become the next-generation "immunohistochemistry" for the aforementioned applications in anatomic pathology laboratories.

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References

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