Evolving Practices of Diagnostic Immunohistochemistry

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It has been more than 6 years since the *Archives of Pathology & Laboratory Medicine* published the special issue on diagnostic immunohistochemistry (IHC) consisting of a series of review articles edited by Jaishree Jagirdar, MD, which was a great, concise, and yet comprehensive review and update on important IHC markers, panels, and diagnostic strategies.1 It has been my great honor and immense privilege to organize this new special issue on IHC, contributed to by pathologists from Geisinger Medical Center (Danville, Pennsylvania) and many expert pathologists from other medical centers. This 2-part special issue features 14 review articles with an attempt to cover IHC automation, standardization of diagnostic IHC, and the role of IHC in diagnosing tumors in major organs and tumors of unknown primary.

This series begins with an article emphasizing standardization of diagnostic IHC in the preanalytic, analytic, and postanalytic phases, with a specific focus on (1) newly proposed guidelines on antibody validation from the College of American Pathologists Pathology and Laboratory Quality Center, (2) testing/optimizing a new antibody and troubleshooting, (3) interpreting and reporting IHC assay results, (4) continuing quality improvement programs, and (5) developing and implementing the concept of best practices in IHC. When it comes to the question of how to implement best practices, emphasis is placed on the evidence-based application of IHC markers to practical scenarios, which includes eliminating unnecessary markers, starting with a small IHC panel and continuing with a second panel only when the first panel leads to an inconclusive result. Two questions should be asked before ordering any IHC assay: “Why should I order this marker?” and “Does it change my diagnosis and patient care?” In addition, some external quality control programs and College of American Pathologists checklists for inspecting a clinical IHC laboratory are outlined. The role of digital pathology in the field of diagnostic IHC is briefly discussed as well.

The second review article compares and contrasts the recent technological advances in new generation automated IHC platforms from all major companies. Some advantages over the prior automated platforms are stressed, including complete walkaway automation, faster speed, use of less reagents, user friendliness, better integration with laboratory information systems, effective waste control, capability for multiplexing, and more reliable and reproducible results.

One of the most frequent and important applications of diagnostic IHC is to assist in working up an undifferentiated neoplasm/tumor of uncertain primary site. The third review article comprehensively reviews some diagnostic strategies and algorithms, updates many recently described diagnostic markers such as GATA binding protein 3 (GATA3), placental S100 (S100P), mammary serine protease inhibitor (maspin), von Hippel-Lindau tumor suppressor gene protein (pVHL), paired box gene (PAX) 8, ETS-related gene (ERG), sal-like protein 4 (SALL4), sex-determining region Y box (SOX) 10, arginase-1, napsin A, special AT-rich sequence-binding protein 2 (SATB2), and cadherin-17, and refines the diagnostic IHC panels frequently used in daily practice.

The remaining 11 review articles are devoted to organ-specific diagnostic IHC. In the lung and pleural review article, p40, desmoglein-3, desmocollin-3, and cytokeratin (CK) 5 are described as the most effective panel of IHC markers for squamous cell carcinoma; hepatocyte nuclear factor 4 α (HNF4α) is demonstrated to be a marker for invasive mucinous adenocarcinoma of the lung; and glucose transporter 1 (Glut1), insulin-like growth factor II messenger RNA–binding protein (IMP3), and cluster of differentiation (CD) 146 are shown to be helpful in differentiating reactive conditions from malignant mesothelial proliferations.

In breast pathology, GATA3 has been reported in several publications to be expressed in most breast ductal and lobular carcinomas, including more than 50% of ER-negative breast carcinomas and metaplastic carcinomas. Ankyrin repeat domain 30A (NY-BR-1) is another newly described marker useful in identifying a breast primary, although in limited publications. Numerous predictive biomarkers have been evaluated, and additional studies are needed to confirm the initial findings.

In genitourinary pathology, PAX8 and PAX2 have proven to be important diagnostic markers for identifying renal cell carcinoma when working on a tumor of unknown primary. ERG is a specific but not sensitive marker for prostatic adenocarcinoma. In contrast, NK3 homeobox 1 (NKX3.1) may become a highly sensitive and specific marker for prostatic adenocarcinoma if additional publications substantiate the initial reports. In limited studies, uroplakin (UP) II has proven to be a more sensitive marker than UPIII for identifying urothelial carcinoma. GATA3 and S100P are useful markers for urothelial carcinoma. A new generation
of germ cell tumor markers, including SALL4, octamer-binding transcription factor 4 (OCT4), lin-28 homolog A (LIN28), SOX2, GATA3, and Nanog homebox (Nanog), has proven to be superior to traditional germ cell tumor markers.

In the field of lymphomas, a group of relatively sensitive and specific biomarkers has been identified for various types of lymphomas: (1) human germinal center–associated lymphoma (HGAL)/GEC12, LIM–only transcription factor 2 (LMO2), and stathmin for follicular lymphoma; (2) lymphoid enhancer–binding factor 1 (LEF1), CD200, and membranous CD160 has been present in virtually all neoplastic cells of B–cell chronic lymphocytic leukemia/small lymphocytic lymphoma; (3) SOX11 expression has been found in almost all cases of mantle cell lymphoma, including both cyclin D1–positive and cyclin D1–negative types, as well as cyclin D1–negative blastoid mantle cell lymphoma; (4) immunoglobulin superfamily receptor translocation–associated 1 (IRTA1) is selectively expressed on the surface of neoplastic cells of extranodal and nodal marginal zone lymphomas; (5) c-MYC could detect increased MYC protein expression by both MYC translocation and MYC overexpression, which could be used as a screening test to select the cases for further investigation using the fluorescence in situ hybridization study; and (6) IMP3 cytoplasmic staining has been found in Hodgkin cells of almost all cases, superior to other IHC markers such as CD15, CD30, PAX5, and multiple myeloma oncogene 1 (MUM1).

In part II, the following review articles will be presented.

In the gastrointestinal, liver, and pancreatobiliary area, 2 new markers, SATB2 and cadherin-17, have been useful in working on gastrointestinal tract tumors. SATB2 appears to be more sensitive than caudal type homeobox 2 (CDX2) and CK20 in identifying colorectal carcinomas; the SATB2 positivity has been reported in CDX2–negative poorly differentiated colorectal carcinomas and medullary carcinomas of the large intestine. SATB2 is frequently positive in colorectal neuroendocrine neoplasms but negative in neuroendocrine neoplasms from other organs. In contrast, cadherin-17 is a positive marker for both lower and upper gastrointestinal adenocarcinomas and neuroendocrine neoplasms. Maspin, S100P, pVHL, and IMP3 have been reported to be a reliable panel of markers to differentiate a carcinoma from reactive ducts in the pancreatobiliary tract, carcinoma being negative for pVHL and positive for S100P, maspin, and IMP3 and reactive ducts positive for pVHL and negative for maspin, S100P, and IMP3. Interestingly, intrahepatic cholangiocarcinoma tends to be positive for pVHL (70% of cases), which can be potentially used in the distinction of a metastatic pancreatic adenocarcinoma from an intrahepatic cholangiocarcinoma.

In liver, a panel of 4 markers, including liver fatty acid–binding protein (LFAB), serum amyloid associate protein (SAA), C-reactive protein (CRP), glutamine synthetase (GS), and hepatocyte nuclear factor 1α (HNF1α), has been proposed for further classifying a hepatocellular adenoma (HCA) into 4 major subtypes: HNF1α–mutated HCA, β-catenin–mutated HCA, inflammatory HCA, and unclassified HCA. Within intrahepatic cholangiocarcinomas, 10% of tumors may also be β-catenin mutated. Arginase 1 is a more sensitive and specific marker for identifying a hepatocellular carcinoma than hepatocyte paraffin 1 (HepPar1) and glypican 3. Arginase 1, like HepPar1, is also positive in normal liver and benign hepatic lesions.

In gynecologic pathology, the loss of p57 expression in the villous cytotrophoblasts and stromal cells is a characteristic feature of complete moles; hepatocyte nuclear factor 1β (HNF1β) is a useful marker for identifying clear cell carcinoma of the uterus and ovary and is usually negative in papillary serous carcinoma; pVHL and kidney injury molecule 1 (KIM-1) have been reported to be helpful in the distinction between clear cell carcinoma of the uterus and ovary from serous carcinoma; forkhead box L2 (FOXL2) and steroidogenic factor 1 (SF-1) have been reported to be sensitive and specific markers for sex cord stromal tumors. SF-1 is also a useful marker for identifying an adrenal cortical neoplasm.

In head and neck pathology, discovered on gastrointestinal stromal tumor protein 1 (DOG1) expression was seen in normal serous acini, mucous acini, and distal intercalated ducts. All acinic cell carcinomas were positive for DOG1, with predominantly apical/luminal membranous staining. Most ductal tumors were negative, but DOG1 immunoreactivity can be seen in mammary analogue secretory carcinomas and pleomorphic low-grade adenocarcinomas. No DOG1 immunoreactivity was seen in salivary duct carcinomas, oncocytomas/oncocytic carcinomas, myoepitheliomas, Warthin tumors, or sebaceous lymphadenoma. GATA3 was positive in 100% of mammary analogue secretory carcinomas and salivary duct carcinomas and a much lower percentage of other salivary gland tumors. Overexpression of p16 is highly correlated with human papillomavirus–positive squamous cell carcinomas of oropharynx and oral cavity; p16 immunostain is considered a very sensitive surrogate biomarker for human papillomavirus infection and has been routinely performed on all squamous cell carcinomas of the oropharynx and oral cavity.

Our group has reported that tumor-associated calcium signal transducer 2 (TROP2) is a potential diagnostic marker for papillary thyroid carcinoma; it appears to have a better diagnostic specificity than the traditional markers, such as CK19, Hector Battifora mesothelial 1 (HBME1) and galec tin 3. Additional studies from others would need to validate our findings.

In the skin review article, androgen receptor and adipophilin expression support the diagnosis of sebaceous carcinoma over squamous cell carcinoma; clonal integration of the newly discovered Merkel cell polyomavirus has been shown in the great majority of Merkel cell carcinomas; claudin-1 and Glut1 are stronger and stain more diffusely in perineuromatous than epithelial membrane antigen (EMA); langerin (CD207) has shown diagnostic sensitivity similar to CD1a but with improved specificity for Langerhans cell histiocytosis; friend leukemia virus integration 1 (FLI1) and ERG have been reported as nuclear markers of endothelial differentiation and are highly sensitive for benign and malignant vascular tumors; positive staining for p63, podoplanin (D2-40), and CK15 favors a primary cutaneous adnexal neoplasm over metastatic adenocarcinoma; and Wilms tumor 1 (WT1) cytoplasmic endothelial expression has been reported in vascular tumors but is lacking in lymphatic and venous vascular malformations.

In soft-tissue pathology, SATB2 has been reported to be a marker for a sarcoma with osteoblastic differentiation; cancer/testis antigen 1 (NY-ESO-1) has been introduced as a marker for myxoid and round cell liposarcoma; mucin 4 (MUC4) has proven to be a marker for low-grade fibromyxoid sarcoma and sclerosing epithelioid fibrosarcoma; NK2 homeobox 2 (NKX2.2) has been shown to be a
more specific marker than CD99 for Ewing sarcoma; mouse double minute 2 homolog (MDM2), cyclin-dependent kinase 4 (CDK4), and p16 are good markers for well-differentiated and dedifferentiated liposarcomas; ERG has been demonstrated to be the most sensitive marker for benign and malignant vascular tumors; subsets of perivascular epithelioid cell tumors and epithelioid hemangioendothelioma are positive for transcription factor E3 (TFE3); succinate dehydrogenase (SDHB) has shown loss of expression in a subset of pediatric gastrointestinal stromal tumors and paragangliomas; signal transducer and activator of transcription 6 (STAT6) has been reported in most solitary fibrous tumors; and loss of integrase interactor 1 (INI-1) is seen in most epithelioid sarcomas and some myoepithelial carcinomas.

Because of the limited scope of this 2-part special issue, applications of IHC in other important areas, such as central nervous system tumors and infectious agents, cannot be included. Isocitrate dehydrogenase 1 (IDH1) has been reported to be a useful marker for differentiating glioma from reactive gliosis. The association of viruses with their associated tumors can be demonstrated, such as Merkel cell polyomavirus in Merkel cell carcinoma, Epstein-Barr virus in Burkitt lymphoma, and human herpesvirus 8 in Kaposi sarcoma and body cavity lymphoma.

Genetic alterations of tumors are traditionally detected by molecular analysis, such as cytogenetics, fluorescence in situ hybridization, or polymerase chain reaction–based assay. With the advances in IHC technologies and rapidly growing knowledge of molecular pathology in the last few years, IHC has entered a new era and is now able to provide specific genetic information about certain tumors. One of the breakthroughs is the generation of mutation–specific antibodies directly against the mutated proteins, such as epidermal growth factor receptor (EGFR) gene L858R and exon 19 deletion mutation in lung adenocarcinoma; BRAF gene V600E mutation in papillary thyroid carcinoma, melanoma, and other cancers; and IDH1 gene R132H mutation in glioma. Specific chromosomal translocations or specific gene rearrangements can be demonstrated through the detection of the related protein products such as B-cell chronic lymphocytic leukemia/lymphoma (Bcl2) in follicular lymphoma, cyclin D1 in mantle cell lymphoma, anaplastic lymphoma kinase (ALK) in lung adenocarcinoma, TFE3 in alveolar soft part sarcoma, and XP11 translocation renal cell carcinoma and c-MYC in Burkitt lymphoma and some postradiation and lymphedema-associated angiosarcomas. The deletion or loss of gene function can also be demonstrated by loss of the expression of related protein products, such as INI-1 in epithelioid sarcomas, renal medullary carcinomas, rhabdoid tumors, and atypical teratoid/rhabdoid tumors; E-cadherin in lobular breast carcinomas and some signet ring cell gastric carcinomas; and mismatch repair proteins (MutL, homolog 1 [MLH1], MutS protein homolog [MSH]2, MSH6, and postmeiotic segregation increased 2 [PMS2]) in microsatellite instability tumors. Gene amplification can be detected through the overexpression of related protein products, such as human epidermal growth factor receptor 2 (Her2) in breast carcinomas and gastroesophageal/gastric carcinomas and MDM2 and CKD4 in well-differentiated liposarcomas/some dedifferentiated liposarcomas.

In summary, there is no doubt that IHC will continue playing an important role in surgical pathology and cytopathology, with additions of more sensitive and specific biomarkers and more well-defined yet smaller IHC panels for each diagnosis and differential diagnosis. Furthermore, more antibodies that provide genetic information or predictive marker information, especially mutation–specific antibodies, are on their way to clinical IHC laboratories.

References