Immunohistochemistry as a Practical Tool in Molecular Pathology

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• ***Context.***—**Molecular genetics is playing an increasingly important role in patient care and pathology practice. Immunohistochemistry (IHC) is a valuable and practical tool employed by most pathologists on a regular basis.**

Objective.—**To highlight select examples of how IHC may be used in the realm of molecular diagnostics.**

Data Sources.—**Select sources on IHC relating to tumor subtyping, hereditary cancer screening, and treatment-response prediction are reviewed. These represent some of the areas in which IHC can be employed by anatomic pathologists to optimize patient care and further inform molecular testing.**

Conclusion.—**In the emerging era of personalized medicine, IHC continues to serve a valuable function, complementing and enhancing other molecular techniques.**

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The past decade has brought incredible innovations to the practice of pathology. At the heart of these innovations is massively parallel genetic (so-called next-generation) sequencing. This is intimately linked with the looming promise of personalized medicine—the practice of delivering individualized treatment, tailored to the disease of individual patients. Despite these technologic advances, most anatomic pathologists do not use next-generation sequencing in their daily practices. These techniques are relegated to research practice or to full-time molecular pathologists who are, at the moment, more removed from clinical care. Nonetheless, immunohistochemistry (IHC) is an ideal tool for pathologists to make valuable contributions in molecular medicine.

Immunohistochemistry is ideally suited to anatomic pathology practice because its use demands mastery of histopathology coupled with expertise in pathobiology. Furthermore, the cost and turnaround time are both low, contributing to the overall utility of this technique. Although more-complicated techniques have dominated discovery and innovation during the past decade, IHC continues to be the best tool for surgical pathologists to practice routine molecular pathology. Traditionally, IHC has been used as a diagnostic tool; however, there are increasing applications of IHC for its predictive and prognostic utility. This article focuses on select examples, highlighting how IHC can be used to practice personalized medicine.

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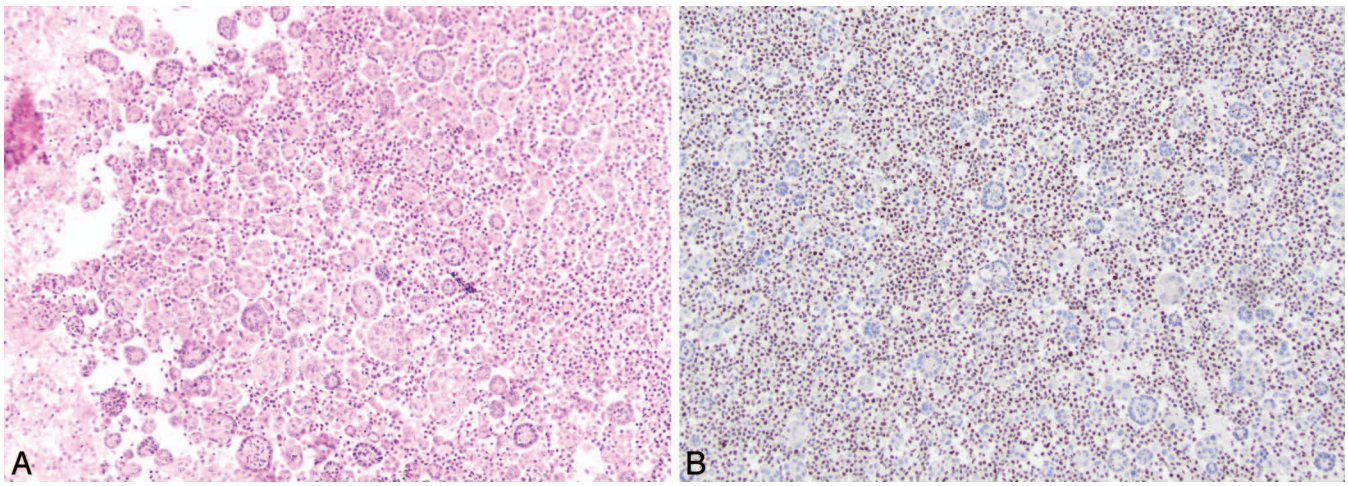
Breast cancer is perhaps the first human malignancy to be treated with targeted therapy, beginning with therapeutic oophorectomy described in the late 1800s.[1] Anti-HER2 (human epidermal growth factor receptor 2) monoclonal antibody treatments began in the 1990s and represented the first use of targeted antibodies in solid tumors.[2] Additional targeted therapies are currently under investigation for the highly aggressive basal-like subtype of breast cancer.[3] Breast cancer was also the first tumor type to have an established, molecular subclassification.[4] These "intrinsic subtypes" of breast cancer are reproducible using multiple methodologies, and each is linked with different clinical behavior and treatment options.[5–7] Fifteen years after the molecular classification of breast cancers, multiple molecular assays are available to guide clinical management; nevertheless, IHC remains the most widely used.[8]

Simple stains for estrogen and progesterone receptors and HER2 are able to provide robust, reproducible, and highly predictive information to clinicians. The addition of Ki-67 may assist in determining the need for chemotherapy in luminal subtypes, and recent reports indicate that this may be clinically useful.[9] Immunohistochemistry directed toward cytokeratin 5 and epidermal growth factor receptor (EGFR) has shown more specificity for the basal-like subtype than a simple "triple-negative" definition, which can be useful for hereditary breast and ovarian cancer screening.[10] This approach may be further refined using the novel markers nestin and inositol phosphate 4-phosphatase.[11] Essentially, a complicated and seemingly impenetrable molecular classification can be approximated with no more than 6 IHC stains. This approach offers an attractive alternative to molecular studies because it is cheap, fast, and within the ability of a competent pathologist.

After use in breast cancer, several other tumor types now feature molecular subclassifications. Gastric cancer has recently, through the Cancer Genome Atlas, been charac-



Example of the utility of BAP1 immunohistochemistry (IHC) for the diagnosis of mesothelioma. A, Cell-block preparation of pleural fluid showing an atypical mesothelial proliferation, suspicious for mesothelioma, however, not diagnostic in the absence of histologic evidence of invasion. B, Demonstrated loss of nuclear signal in the malignant cells. Note the positive staining in background inflammatory cell nuclei serving as an internal positive control. This staining pattern is highly specific for mesothelioma (hematoxylin-eosin, original magnification ×10 [A]; BAP1 IHC ×10 [B]). Photomicrographs provided by J. Wright, MD (St. Paul's Hospital) and A. Churg, MD (Vancouver General Hospital).

terized into 4 molecular subtypes (Epstein-Barr virus–positive, microsatellite unstable, genomically stable, and chromosome instability).[12] Although these subtypes were derived using comprehensive molecular profiling, the most clinically relevant features, including treatment and hereditary implications, can be reproduced using (1) mismatch repair (MMR) IHC, (2) HER2 IHC, (3) E-cadherin IHC, and (4) Epstein-Barr virus in situ hybridization (not technically IHC, but a technique based on formalin-fixed, paraffin-embedded tissues/glass slides and morphology nonetheless).[13–18] All these stains may be readily performed and interpreted in most anatomic pathology laboratories. Similarly, endometrial carcinoma has been subject to a clinically relevant molecular classification by the Cancer Genome Atlas.[19] With endometrial carcinoma, as well, the copy-number high (serouslike), copy-number low (endometrioid), hypermutated (MMR deficient), and ultramutated (polymerase [DNA-directed]–ε; POLE) can be reproduced using p53 and MMR IHC, leaving only judicious sequencing of *POLE* to discriminate between the final 2 subtypes.[20–23]

Mismatch repair IHC not only forms the cornerstone of IHC/molecular classification of gastric and endometrial carcinomas but also has carved a role in hereditary cancer screening for Lynch syndrome.[24] The approach is further complemented by the addition of IHC for BRAF V600E, which also provides additional predictive information.[25,26] Reflex testing by IHC offers superior screening to previously used clinical tools.[27,28] This strategy has been pivotal in the improved detection of sentinel Lynch-associated cancers (estimated at >3000 Lynch-associated colorectal cancers per year in the United States).[29] Mismatch repair IHC not only helps screen for Lynch syndrome but also can guide direct gene sequencing to make this process more cost and time efficient.[30] Furthermore, the widespread use of MMR IHC has seen its off-label characterization as a predictive biomarker for both chemotherapy and targeted therapies.[31,32]

Predictive and hereditary gene sequencing can be aided and directed by IHC, not only for MMR but also in multiple other settings. An excellent example is provided by gastrointestinal stromal tumors (GISTs). These tumors are driven by mutations in *KIT* or platelet-derived growth factor receptor–α (*PDGFRA*).[33,34] Sequencing these genes can help guide targeted therapy and identify hereditary GISTs.[35] Immunohistochemistry for KIT (CD117), discovered on GIST 1 (DOG1), and for succinate dehydrogenase complex, subunit B (SDHB), offers a valuable triage tool to direct gene sequencing and can save on both cost and turnaround time in this tumor type.[36] In these examples, IHC can be used to simultaneously provide patient triage for hereditary cancer screening, guide genetic-sequencing studies, and to directly inform therapeutic decision-making.

Immunohistochemistry has a number of uses in which it can predict genetic alterations. In breast and gastric cancer, HER2 overexpression by IHC is highly predictive of underlying *ERBB2* amplification.[2,16] In lung and other malignancies, aberrant expression of anaplastic lymphoma receptor tyrosine kinase (ALK) or ROS can be detected by IHC because of rearrangements involving the *ALK1* and *ROS1* genes.[37,38] In solitary fibrous tumors, signal transducer and activator of transcription 6 (STAT6) IHC is, again, highly predictive of the pathognomonic NGFI-A binding protein 2 (*NAB2*)–*STAT6* fusion.[39,40] In mesothelioma, and other malignancies, loss of BRCA-associated protein 1 (BAP1) immunoreactivity is predictive of genetic inactivation by mutation, deletion, or both (Figure, A and B).[41,42] In these examples, IHC can reduce the need for more-complex tests, such as fluorescence in situ hybridization; improve the efficiency of both predictive biomarker testing; and enhance diagnostic accuracy.

More recently, improvements in monoclonal-antibody generation have led to the ability to detect single amino acid changes. This has directly translated into the ability to diagnose specific genetic mutations using IHC. The common oncogenic driver BRAF V600E can now be identified routinely by pathologists and has a variety of clinical applications in melanoma, thyroid carcinoma, colorectal carcinoma, and other tumor types.[43–45] In lung cancer, IHC can also be employed to detect the 2 most-common mutations in *EGFR*.[46] This can be used to provide direct genotype results in a fraction of the time required for traditional genetic testing. Similarly, antibodies directed

toward the R132H mutation in the isocitrate dehydrogenase 1 (IDH1) protein have proven useful in the diagnosis of gliomas.[47]

Explored here are a few examples of the use of IHC in molecular pathology to subclassify tumors, such as in breast, gastric, and endometrial cancers; to screen for hereditary cancers, such as with MMR IHC and Lynch syndrome; and to diagnose underlying genetic changes, including amplifications (eg, *ERBB2*), translocations (eg, *STAT6*), and point mutations (eg, *BRAF* V600E). This is not meant as an exhaustive review of the molecular applications of IHC but rather to highlight the effectiveness of IHC as a molecular tool through pertinent examples.

In the current landscape of surgical and molecular pathology, IHC remains one of, if not the most, valuable tool at our disposal. Immunohistochemistry complements, and at times supersedes, DNA sequencing or gene-expression profiling. Good working knowledge of IHC is perhaps one of the most important attributes of the modern molecular pathologist. This underscores the importance of histopathology and glass-based morphology for the practice of molecular pathology.

For the anatomic pathologist, IHC provides the perfect medium to deliver valuable molecular information at the time of diagnosis. Anatomic pathologists represent a crucial hub in patient-care pathways, enabling them to deliver some of the highest impact in molecular and personalized medicine during their daily sign out.

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