Next Generation Immunohistochemistry (IHC):

A Window Onto the Molecular Biology of Tumors

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The seminal work in the field of IHC commenced with Albert Coons in the 1940s, and culminated with Clive Taylor and colleagues in the 1970s, as immunohistochemical methods were devised and systematically refined to identify specific cell types in tissue sections. Cell type analysis, in fact, has driven IHC development since the1980s and 1990s as monoclonal antibody technology has permitted development of reagents specific for cell types, many of which have proved to be surrogates for tumor diagnoses (e.g., TTF-1 for lung adenocarcinoma, SALL4 for germ cell tumors, CD20 for B cell lymphoma, etc.). In many cases these markers have complemented histology, but in other cases,e.g., when the tumor is poorly differentiated, they may be surrogates. More recently, and looking towards the future, 'next generation' IHC will be providing a window onto the molecular alterations which underlie cancers. The major genetic alterations in human cancers include mutation, translocation, deletion, amplification, and alteration in gene expression, e.g., through methylation. Each one of these genetic

alterations has a corresponding protein alteration that can be identified by next generation immunohistochemistry. The latter employs a different set of non-cell type specific antibodies. For example, mutation can result, depending upon the gene involved, in loss of protein expression (e.g., mismatch repair protiens MLH1, MSH2, PMS2, and MSH6 in colon cancer; E-cadherin in lobular breast cancer), abnormal protein accumulation (e.g., p53 in ovarian and other cancers), or abnormal localization of the protein (e.g., beta-catenin in abdominal fibromatosis). In some cases, mutation can result in abnormal localization of other proteins with which it forms a complex (e.g., abnormal localization of beta-cateinin in the presence of APC mutations in colonic adenocarcinoma). Mutated proteins may also be identified using monoclonal antibodies to mutation specific epitopes (e.g., BRAF V600E in melanoma and other tumors; IDH1 R132H in low grade gliomas). In most cases, the use of next generation IHC is faster and less expensive than molecular analysis, e.g., sequencing., and can provide more information, particularly through the simultaneous preservation of morphology. Loss of expression of protein, however, can result not only from mutation, but also gene deletion and methylation (e.g., INI1/SMARB1 loss of expression in rhabdoid tumors, epithelioid sarcoma, and other tumors). The presence of chromosomal translocations that are specific for selected tumors can also be identified by next generation IHC, through localization of a fusion protein, or identification of a protein not normally expressed. Examples include identification, with antibodies to FLI1, of the presence of the t(11;22)(q24;q12) translocation of PNET/Ewings sarcoma by identifyng the EWSR1-FLI1 fusion protein. Similarly, antibodies to ALK can identify the presence of the t(2;5)(p23;q35) translocation of anaplastic large cell lymphoma as well as the chromosome 2 inversion

which defines in a small subset of lung adenocarcinoma that can be treated with crizotinib, and antibodies to TFE3 can identify the ASPL-TFE3 fusion gene that results from the der(17)t(X;17)(p11;q25) translocation that characterizes alevolar soft part sarcoma. In the case of ALK in lung adenocarcinoma, immunohistochemistry can help make more efficient screening for the presence of ALK chromosomal translocations and result in more judicious use of fluorescence in situ hybridization. Identification by IHC of protein overexpression that is a direct consequence of gene amplification can serve as a surrogate marker for the latter in cases of breast cancer (HER2), liposarcoma (MDM2, CDK4), lymphoma (bcl-2), and lung cancer (EGFR). The use of IHC can also serve as link between the molecular pathology and histology, and can be a tool to extend the histologic range of tumors once the molecular basis is understood (e.g., ALCL, where new histologic variants, such as small cell monomorphic variant, can be defined by the presence of ALK expression). In the future, the pathologist will be playing a critical role in the determination of appropriate treatment for cancers, and next generation IHC can provide an important tool for the identification of molecular alterations that not only define specific tumors, but provide a basis for their successful treatment.