A Challenge for the Diagnostic Immunohistopathologist

Adding the CD Phenotypes to Our Diagnostic Toolbox

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As medical knowledge grows and demands for subspecialty expertise increase, the practice world of the diagnostic anatomic pathologist risks being compartmentalized into 2 functional knowledge domains: the domain in which practicing pathologists have confidence in their knowledge, and the rest. One knowledge domain in pathology that has seen an exponential growth in information, the clinical relevance of that information, and the demand for subspecialty expertise is hematopathology. A consequence is that fewer generalist surgical pathologists are comfortable with contemporary hematopathology diagnostic approaches. A major component of current hematopathology practice is based on interpreting patterns of expression of the cluster designation (CD) antigens, of which there are more than 250. The present issue of the Journal and a previous issue contain articles that report the use of CD immunochemical analysis, by flow cytometry1 or by immunohistochemical analysis,2 in solid tumor diagnosis. These articles exemplify the value of being aware of what goes on outside the narrow box of a subspecialty knowledge domain.

Historic Use of CD Antigens

The CD antigens are cell membrane–localized antigens that identify subsets of hematolymphopoietic cells. The CD antigens (characterized in detail at http://www.ncbi.nlm.nih.gov/prow/guide/45277084.htm) were first identified as lymphocyte cell surface molecules against which monoclonal antibodies were generated. Developmentally and functionally distinct lymphocyte subsets were distinguished by their patterns of CD expression. As monoclonal antibodies were generated against lymphoid antigens, a process of vetting antibodies for CD naming was established. This process involves distributing monoclonal anti–cell membrane antibodies to different laboratories, which confirm localization and patterns of expression of the candidate CD antigen using primarily immunologic techniques, ie, competition immunoenzyme assays, Western blot analyses, and flow cytometry. Although the initial focus was on lymphocyte cell membrane antigens, the effort has expanded to the point that antibodies need not be expressed by lymphoid cells to merit candidacy as a CD marker.

Clinical Applications

Diagnostic Applications

The characterization of patterns of CD expression by lymphoid cells, using flow cytometric and immunohistochemical techniques, is a powerful approach for diagnosing lymphoproliferative processes. By CD immunophenotyping of proliferative lymphoid cells, lymphomas can be assigned to subcategories that are managed in clinically different manners. For example, some B-cell lymphomas now are treated with anti-CD20 antibody therapy.3 Lymphomas and leukemias can be diagnosed based not only on the predominant cell population but also on anomalous and/or overexpression of specific CD antigens. For example, some B-cell lymphomas overexpress CD5, and some acute myelogenous leukemias anomalously express CD56, which is not normally expressed by myeloid blasts.
The technology of the assays in which these antibodies are used continues to evolve. For example, simultaneous 8-color flow cytometry is used routinely in a number of diagnostic hematopathology laboratories, and more than 12 differentially fluorochrome-labeled cell types are being separated in research laboratories. A consequence of these developments is the ability to identify minor populations of malignant cells.

This approach of diagnosing neoplasms based on patterns of CD expression includes the diagnosis of solid tumors. One of the first CD antigens to be studied in solid tumors was carcinoembryonic antigen (CD66e). This protein was first used as a serum marker for recurrent colonic adenocarcinoma. Because CD nomenclature has not been used consistently, anatomic pathologists may not have always been aware of the value of these monoclonal anti-CD antibodies for making histopathologic diagnoses. For example, in addition to carcinoembryonic antigen (CD66e), CD15, also known as Leu-M1, helps to distinguish adenocarcinomas (usually CD15+) from mesotheliomas (CD15–), and CD30 distinguishes embryonal carcinoma (CD30+) from other germ cell tumor components.

Research Applications

Antibodies to cell membrane antigens, when tagged with fluorochromes, have served as valuable research tools. Put through a flow cytometer, CD-labeled cells can be selected from a heterogeneous population of cells for detailed characterization of genotype and phenotype. By using anti-CD44 and anti-CD57, prostate basal cells and luminal secretory cells have been sorted and epithelial cell-type specific complementary DNA (cDNA) libraries have been generated. Another more recent technique for using CD expression to separate cells has used anti-CD antibodies bound to magnetic beads. Relatively pure populations of CD34-expressing bone marrow stem cells have been obtained by using this approach.

Ever greater certainty of the cell type selected for molecular characterization has resulted from using these antibodies to label cells in tissue sections. By using a fluorescence microscope equipped with a laser capture device, labeled cells can be isolated. Gene expression analysis of these cells can be carried out by reverse transcription of RNA and amplification to produce an amount of cDNA that is sufficient for probing expression microarrays.

Since tumor behavior likely is dependent on the cell types that compose a cancer, identification of these cell types and calculation of their ratio based on a panel of CD antibodies, such as we used in immunophenotyping the prostate, may lead to diagnoses that include prognostic information specific to that CD subtype of tumor. By using CD markers, specific cancer cell types also may be isolated for analysis by genomics and proteomics tools. These analyses promise to lead to a molecular understanding of the cancer process. Such analysis is not limited to cancer cells. It can be applied to study normal processes of cellular differentiation and to better understand cellular phenomena of nonneoplastic disease.

Potential Pitfalls and Problems

Based on both nomenclature and the history of the CD system, assumptions that are not necessarily correct have been made. The fact that most CD antigens are expressed by hematolymphoid cells does not mean that nonhematolymphoid cells may not express these antigens. An example of how misleading the assumption might be that expression of a CD antigen equates with cell lineage was the report that expression of CD57 by undifferentiated small cell lung carcinoma is evidence that this tumor originates from bone marrow macrophages that have migrated to the lung. Subsequent work has shown that these antigens, including CD57, are expressed by different types of epithelial cells, both normal and neoplastic.

One might mistakenly assume that each CD antigen is a unique gene product. Although a majority of these antigens are encoded by gene transcripts, there are exceptions. For example, CD15 is a carbohydrate antigen that is a post-translational product of membrane-associated protein(s) expressed on a variety of cells, including a subset of T cells, normal and neoplastic glandular cells, and Reed-Sternberg cells of Hodgkin lymphoma.

Expression of an antigen that is a target for defined molecular therapy does not predict the response of all cells expressing that antigen to the specific reagent. For example, CD117 (or c-kit), is a transmembrane tyrosine kinase receptor that, when activated in gastrointestinal stromal tumors, can be inactivated by the drug imatinib mesylate (Gleevec). Gastrointestinal stromal tumors respond to treatment with this drug. A mistaken assumption would be that all tumors composed of cells expressing CD117 would respond to imatinib mesylate. That assumption is inaccurate. The forms of c-kit that respond to imatinib mesylate are activating mutations.

Patterns of CD expression may be subject to laboratory artifact. Consequently, the CD profile of a cell population may not be representative of the in vivo, “true” profile of that cell type. For example, there is an alteration to the CD profile when prostate tissue is explanted into short-term cell culture. This finding is of potential clinical importance if the CD profile of a tissue sample changes substantially during the time after removal from the patient and preparation for analysis.
Future Challenges and Possibilities

Disease Prognostication

Differential expression of specific CD molecules provides prognostic information. For example, melanomas that metastasize express the neutral endopeptidase CD10.18 Loss of CD10 expression is an early event in prostate cancers.19 CD26+, histologically “benign” thyroid neoplasms are likely to metastasize; most thyroid neoplasms lack CD26.20 And, non–small cell lung carcinomas that contain CD13+ tumor cells are associated with a worse prognosis than tumors that are free of CD13+ cells.21 Although the actual functional role of these proteins is unknown, experiments have demonstrated properties that may explain their malignant state. For example, CD26 has a role in the migration of fibroblasts on tissue matrix by binding fibronectin.22

Targeted Therapy

CD molecules can be targeted by CD-specific molecules. In addition to using anti-CD20 antibodies to treat some B-cell lymphomas,3 CD molecules can be targeted by specific inhibitory drugs. For example, diprotin A, isolated from Bacillus cereus, inhibits CD26.23

Closing Comment

Our discussion has focused on the usefulness and complexity of CD phenotyping of solid tumors as a set of challenges to the anatomic pathologist. There is another perspective. The anatomic pathologist who knows about CD phenotyping tissues and solid tumors can make invaluable contributions to the clinical diagnosis and therapy of patients. Pitfalls such as those described herein can be avoided. And inappropriate and ineffective use of molecular therapy targeted to specific CD antigens can be prevented.

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References