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Review Article

Immunohistochemical approaches to the diagnosis of undifferentiated malignant tumors

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Abstract Undifferentiated malignant neoplasms are a daunting diagnostic problem for anatomical pathologists, calling for a tour de force in morphological skill, clinicopathologic correlation, and application of adjunctive laboratory studies. The most useful approach to these lesions begins with generic classification into 1 of 4 histologic categories: small round cell; spindle cell; large polygonal cell (epithelioid); and pleomorphic neoplasms. Once that step has been accomplished, one can systemically apply corresponding groups of antibody reagents in immunohistologic studies and interpret the results in an algorithmic fashion. This review presents the tumor markers that are the most useful in this contextual approach, as well as the specific algorithmic structures that can be applied to the 4 specified tumor groups. Other selected problems in the diagnosis of morphologically ambiguous tumors are considered as well.

Keywords: Undifferentiated malignant neoplasms; Immunohistology; Carcinomas; Melanomas; Lymphomas; Sarcomas; Mesotheliomas; Algorithmic diagnosis

1. Introduction

Not uncommonly, pathologists are confronted with neoplasms that have few distinguishing microscopic characteristics and, therefore, appear to be "undifferentiated." The differential diagnostic considerations in such cases are many, and special studies are almost always necessary to reach a definite conclusion. Making a diagnostic separation between 2 differentiated but histologically similar neoplasms represents an additional challenge in surgical pathology that requires ancillary laboratory analyses. This presentation will address approaches to these dilemmas that are particularly suited to the use of immunodiagnosis. The following discussion is not intended to be exhaustive or all-inclusive; rather, the author's aim is to provide a framework for the contemporary use of immunohistochemistry in the interpretation of solid tumors.

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2. Reagents for the evaluation of undifferentiated neoplasms

An extensive array of antibodies is available to the surgical pathologist to facilitate characterization of tumors without histologically specific features. A highly select panel of immunostains, based on the histopathologic impression of the tumor, can be extremely useful to narrow the diagnostic considerations, if not definitively identify the tumor. The antibodies used for this purpose in our laboratory are described briefly below.

2.1. Immunohistochemical reagents

2.1.1. Intermediate filaments

Cytokeratins are constituents of the intermediate filaments (IFs) of epithelial cells expressed in various combinations depending on the epithelial type and the degree of differentiation. This class of IFs remains among the most commonly studied determinants in immunohistochemistry. Cytokeratin positivity helps corroborate a diagnosis of carcinoma and typically rules out the possibility of sarcoma, malignant lymphoma, or melanoma [1,2]. Possible exceptions include synovial sarcoma, chordoma, Ewing's sar-

coma, and epithelioid sarcoma; some smooth muscle tumors may also react for keratin.

Monoclonal antibodies are now available to a wide range of keratin proteins (40-67 kd). To maximize cytokeratin detection, proteolysis or microwave-mediated epitope retrieval in citrate buffer is mandatory before application of primary antibodies to rehydrated paraffin sections. Because, in most cases, the question is whether any cytokeratin is present in a given neoplasm, combinations or "cocktails" of monoclonal antibodies may be prepared to evaluate the widest range of kilodalton weights.

Antikeratin cocktails are the most useful in the diagnosis of poorly differentiated epithelial tumors, but monospecific keratin antibodies also have distinct advantages in selected circumstances. For example, Merkel cell carcinoma of the skin—an example of a small round cell undifferentiated malignancy—regularly expresses keratin 20, whereas its differential diagnostic simulators generally do not [3].

Of course, one must be assured that the anticytokeratin preparations being used are specific. This point is particularly important with respect to the immunodetection of IFs as a group (cytokeratin, vimentin, desmin, glial fibrillary acidic protein [GFAP], and neurofilament protein) because all share a common peptide sequence [4].

Vimentin is an IF that is present in most mesenchymal neoplasms and a variety of other classes of neoplasms (eg, endometrial carcinoma) [5-7]. It is highly valuable as a marker of adequate tissue fixation and processing, in which positive and appropriate vimentin staining provides certainty of the ability of the tissue to react with antibodies in general [8]. Desmin is another IF present in mesenchymal lesions, found principally in myogenous tumors such as rhabdomyosarcoma and leiomyosarcoma [9].

The neural IFs include neurofilament proteins and GFAP. The first of these is restricted to neuronal and neuroendocrine cellular proliferations [10]; however, it is suboptimally preserved in formalin-fixed specimens. Glial fibrillary acidic protein immunoreactivity characterizes glial neoplasms such as astrocytomas and ependymomas [11]. Expression of this reactant appears to be maintained even in poorly differentiated tumors of the nervous system.

2.1.2. CD45

CD45 is a surface antigen expressed by virtually all hematolymphoid proliferations, and monoclonal antibodies for this marker are reliably specific [12,13]. The use of CD45 is enhanced by concomitant staining with panels of antibodies to cytokeratin and S100 protein. These reagents are helpful in the resolution of such problems as whether a polygonal or small cell undifferentiated lesion is a carcinoma, lymphoma, or melanoma.

2.1.3. Epithelial membrane antigen

Since its description in 1979 [14], epithelial membrane antigen (EMA) has become a widely used determinant in diagnostic immunocytochemistry; it is also known as MUC-1 Epithelial. membrane antigen represents a complex membrane glycoprotein originally isolated from milk fat globules; it is unrelated to the keratin family [15-17]. If membrane-based immunoreactivity is required to define a positive result, monoclonal antibodies to this discriminant are useful in determining the epithelial nature of undifferentiated tumors [18]. The tissue distribution of EMA is largely similar to that of keratin, with some caveats; in particular, not *all* epithelia are positive. Specifically, hepatocellular carcinomas, adrenocortical carcinomas, and malignant germ cell neoplasms lack EMA [16,19]. Also, EMA may be seen in some nonepithelial lesions. Large cell anaplastic lymphoma, plasmacytoma, some T-cell lymphomas [20,21], epithelioid sarcoma, synovial sarcoma, and meningioma may also show potential EMA reactivity [22].

2.1.4. MOC-31

MOC-31 is a 41-kd glycoprotein that is cell membrane-based; it is widely distributed in epithelial cells and tumors in many tissue sites [23,24]. Monoclonal antibodies to this determinant have most often been used in the diagnostic distinction between serosal adenocarcinoma and mesothelioma (which typically lacks MOC-31) [25], but they also fail to label a selected small group of epithelial malignancies that includes hepatocellular carcinomas, germ cell tumors, and renal cell carcinomas [26].

2.1.5. Placental alkaline phosphatase

The isoenzyme of alkaline phosphatase that is expressed by the normal placenta (PLAP) is also evident as an oncofetal antigen in some genitourinary, gastrointestinal, and pulmonary carcinomas [19,27]. Moreover, it is nearly universally seen in germ cell tumors [28-30]. Immunostains for PLAP therefore have their greatest application in separating germ cell neoplasms from somatic tumors. We have found anti-PLAP to be an extremely useful screening reagent for possible germ cell differentiation in undifferentiated tumors.

Anti-PLAP consistently identifies both seminoma and embryonal carcinoma [29,30]. Both of those tumors differ from most other epithelial malignancies in that they generally lack EMA reactivity. Seminoma also is devoid of keratin reactivity in 80% of cases, whereas embryonal carcinoma is keratin-positive. CD30 is helpful in the latter distinction because it is present in embryonal carcinoma and is only rarely if ever seen in seminoma [30,31]. In contrast to germ cell tumors, most PLAP-positive somatic tumors uniformly express EMA and lack CD30 [19]. Therefore, a panel of stains that includes pankeratin, EMA, CD30, and PLAP is useful in distinguishing among these pleomorphic neoplasms.

2.1.6. Carcinoembryonic antigen

Carcinoembryonic antigen (CEA) has enjoyed its greatest recognition in clinical medicine as a serologic indicator of the growth of colorectal cancer. Immunohistochemically, CEA is strongly expressed in colorectal adenocarcinoma, but it may be found in many other epithelial tumors as well