USE OF DIAGNOSTIC ALGORITHMS IN IMMUNOCYTOCHEMISTRY


"The adventurous physician goes on, and substitutes presumption for knowledge. From the scanty field of what is known, he launches into the boundless region of what is unknown."

Thomas Jefferson (1763-1826)

One of the most challenging aspects of diagnostic histology is to determine the site of origin of poorly differentiated metastatic deposits. While many factors contribute to the reliability and validity of this diagnosis, the most critical factor is the standing and credibility (1) of the pathologist performing the examination. A histologic diagnosis is an opinion based upon experience and attained with a varying degree of confidence. In instances of uncertainty, a wise pathologist will consult one or more colleagues and a diagnosis may be reached by consensus; an exercise in the democratic process that is equally as fallible in pathology as in the political arena. A wise pathologist may also resort to one or more of several ancillary techniques designed to provide additional information upon which a rational diagnosis may be founded.

Circumstantial evidence is admissible, such as the known presence of tumor elsewhere, or a social or family history, or even the reported statistical incidence of different tumor types at the site in question; but such evidence should be considered last, rather than first, in reaching a diagnosis. If orthodox light microscopy alone does not permit a confident diagnosis, then the pathologist may search for microstructural clues, such as the presence of "prickles or intracellular bridges" (presumptive squamous carcinoma) or intracellular striations or fibrils (presumptive rhabdomyosarcoma), recognizing that faith and imagination, fired by circumstantial evidence, play large parts in determining whether or not these features are perceived in a particular tumor. Electron microscopy may also be of value in providing ultrastructural evidence of squamous differentiation, muscle cell differentiation, or glandular differentiation, but often the pathologist does not have material available for electron microscopic studies; even if material is available the time for processing and examination in most institutions produces an inordinate delay in reaching the final diagnosis. Furthermore, relatively few surgical
pathologists are versed in the art of electron microscopic interpretation, and only major centers have electron microscopists with experience of using the technique for tumor diagnosis.

Histochemical stains (2,3) have proven of value in some instances, such as in the demonstration of PAS-positive material or mucins in suspected adenocarcinomas, or in the use of "melanin" (silver) stains in suspected melanoma. However, these types of histochemical stains lack true specificity in terms of defining particular cell or tumor types, and can do no more than sway the pathologist in one direction or another. More specific histochemical techniques, dependent upon the reaction of substrate to reveal the presence of a particular enzyme within a cell series, are notoriously susceptible to the adverse effects of fixation or paraffin embedding and thus are not applicable to routinely processed tissues.

The advent of immunocytochemistry promises tangible advantages in the precise definition of cell types that escape morphological recognition. It must be admitted that the range of antibodies currently available, although extensive (e.g. from Ortho Diagnostics, Carpinteria, California), is inadequate for the purpose in hand, and that in the use of immunohistochemical methods for classification of tumors we stand at the beginning, but it is a beginning full of promise.

Table 1 lists one approach to the use of immunohistochemical methods in facilitating diagnosis of unknown tumors. The antigens listed are those for which there is reasonable evidence in the literature concerning their general utility and the reproducibility of staining. A number of other antibodies of equal or greater potential value have been described (e.g. anti-pancreatic cancer antigen, anti-lung cancer antigen) (4-11), but data relating to their usefulness are either nonexistent, or limited, or subject to such controversy that no recommendation can at present be given. Even with the antibodies/antigens described, it must be remembered that not all antibodies of a named specificity (e.g. anti-keratin) in fact have identical specificity [many antibodies (antisera) will show overlapping specificities—only monoclonal antibodies may be said to have identical specificity and then only if demonstrated experimentally or if derived from the same clone.]

An initial approach in our laboratory is to screen tumors of unknown origin with monoclonal (or conventional) antibodies against carcinoembryonic antigen (CEA), keratin and vimentin (Lab Systems, Inc., Chicago, Illinois). The great majority of carcinomas will show positivity of at least a proportion of the carcinoma cells for CEA or keratin or both (Table 1). Squamous carcinomas show a propensity for more extensive positivity with anti-keratin antibody. Adenocarcinomas more frequently are positive with anti-CEA, but may show positivity for cytoskeletal keratin filaments if suitably processed (especially if trypsinized). The observation of this "overlap" precludes the formulation of a dogmatic approach to diagnosis. Vimentin positivity is seen in sarcomas, carcinomas being uniformly negative (12). One residual problem concerns the antibodies themselves; clearly not all anti-CEA antibodies