Histopathology as a Predictive Biomarker: Strengths and Limitations

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Expanded Abstract

Cancer is a physical alteration of the relation of cells and their tissues resulting in aberrant social organization. These alterations are detected as masses (tumors) or, in the case of leukemia, as an abnormal number of white blood cells. However, many inflammatory lesions also form masses, and not all neoplasms are malignant. Therefore, histological criteria of malignancy are used for the diagnosis of cancer. Histopathology is the sine qua non of cancer diagnosis. Any other putative marker of cancer must be validated by histopathologic examination.

Grading and staging of cancers are used to predict the clinical course and outcome of individual cancers. Grading of cancer is based on the histological criteria of the neoplasm including the degree of deviation from normal of tissue architecture and the differentiation and proliferation of individual cells. Extensive studies have correlated these microscopic characteristics with clinical outcome. Grading of most neoplasms, whether invasive or preinvasive, is useful in prognosis and consequently in therapy decisions. Staging, in most neoplasms, is based on the local extent of tissue involvement and/or the detection of the neoplastic cells in distant sites with microscopic confirmation.

The molecular revolution has provided great insight into the genetic alterations leading to cancer and has given hope, in some quarters, that molecular techniques will supplement, or even supplant, microscopic examination. Each biomolecule can now claim its own view of organismic disease states with the suffix “-omics” (genomics, metabolomics, proteomics, glycomics, lipomics, etc.), and each promises to perfect biomarkers as the knowledge base evolves. The discovery and characterization of prostate-specific antigen (PSA) has been one of the great triumphs of this approach. However, some studies indicate that PSA lacks the specificity and sensitivity needed for a biomarker (1–3). The advocates of “systems biology” are diligently using their tools to find the next biomarker. However, their technologies will be validated using the gold standard of cancer diagnosis, microscopic histopathology.

Early detection, without doubt, has had a major impact on the successful treatment of cancer. The improved cure rates of diseases such as cervical and breast cancers are indications of the impact of early detection on treatment.

Early detection of most solid cancer involves the recognition and understanding of precancers known as carcinoma-in-situ or intraepithelial neoplasia. These foci of atypical cells are considered the precursors to malignancy. Students of specific cancers have identified apparent morphological continua that suggest a sequential acquisition of characteristics leading from normal to malignancy (cancer) (4–14).

Although histopathology has successfully detected and characterized these early lesions, these studies also illustrate the limitations of histopathology as a predictive biomarker. Our current concepts of neoplastic progression are largely based on the “multiple genetic hit” hypothesis and the “linear sequential acquisition” models of neoplastic progression. Although somewhat successfully applied to colon cancer, the successive acquisition model has been less predictive in cervical, prostate, and breast cancer, where the relation between “low-grade” intraepithelial neoplasia and invasive cancer has been questioned (15–17). Cervical intraepithelial neoplasia is an excellent example where a specific viral infection may be required to develop a high-grade, progressive in-situ lesion (17). Thus, alternative models of neoplastic evolution have been proposed in which there are several lineages that progress in parallel. These are found to fit observed histological (18) and molecular observations (19) better than a simple linear acquisition model.

Mouse models of tumor biology are informative. The insertion or manipulation of genes that are associated with human malignancy has resulted in murine tumors that mimic the

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Abbreviations used: DCIS, ductal carcinoma in situ; GEM, genetically engineered mouse; PSA, prostate-specific antigen.
histological and cytological organization of human cancers in remarkable detail (20). Further, early preinvasive lesions have been identified in most models, implying a neoplastic progression consistent with the linear sequential acquisition of neoplastic traits (21). A problem with genetically engineered mouse (GEM) models of cancer is the diffuse susceptibility of the target organ resulting in multiple concurrently progressing neoplasms. Thus, assessment of stage and grade is complicated and sometimes impossible for a given neoplasm.

The biology of neoplastic progression in the mouse can be studied using tissue transplantation. The biology of “preneoplasia” was first studied in the mammary gland using virus-induced hyperplastic nodules (22). A linear sequential model consisting of “nodulegenesis” followed by “tumorogenesis” was proposed and became the accepted view when applied to human breast cancer (7,8,13,14,22). Numerous “preneoplastic” hyperplastic outgrowth (HPO) lines have been studied (22). The HPO lines can be classified by their relative risk of malignant transformation. High- (100%) and low- (3%) risk HPO have been generated (23,24). The relative risk of malignant transformation cannot be correlated with any morphological pattern or cytological characteristic. Extensive searches for biological, cytochemical, and molecular markers have failed (25). The reproducible natural history of the transplanted HPO is the only reliable measure of biological potential and is the operational test for premalignancy.

The test-by-transplantation has been extended to GEM models by several laboratories (26,27). Studies of GEM with the Polyoma Virus middle T(PyV-mT) transgene have proven that GEM have biologically premalignant foci of mammary intraepithelial neoplasia (MIN) that can be identified in situ, isolated, and serially transplanted in gland-free fat pads (28). GEM MIN, like its human counterpart ductal carcinoma in situ (DCIS), is morphologically and biologically heterogeneous (26,28,29). However, the morphologic grading criteria in GEM MIN have not proven to be predictive of biological potential. In brief, outgrowths from MIN (MIN-O) initiated by the same oncogenes, isolated from the same animal and the same mammary gland, have different morphologies and different biological potentials (28). However, serial transplantation of small fragments of a given MIN-O line results in the same latency period and the same metastatic rate in each generation. This suggests that the biological potential is inherited and already encoded in progenitor cells unrecognized by microscopic examination. Identification and characterization of these mysterious cells may permit predictive precision. Intervention and prevention studies indicate that MIN-O growth can be profoundly influenced by diet and chemotherapy (30). However, tumors still arise from the treated MIN-O suggesting selection of resistant malignant populations. Thus, the limitation of histopathology: “What you get is not what you see.”

The natural history of GEM model precancers does not fit the standard “multigene hit, sequential acquisition” models of neoplastic progression. Their natural history is more consistent with the “parallel” models (18,19), suggesting the existence of a population of cancer progenitor cells leading to separate branched but parallel development of either intraepithelial or invasive tumors. If validated, the “parallel” model would explain why the linear models of DCIS and prostatic intraepithelial neoplasia have been discarded in clinical medicine and why histopathology has failed as a predictive marker (15,16). If parallel “coevolution” of in situ and invasive neoplasia can be validated, the implications can be immediately translated to clinical medical practice where therapeutic decisions for DCIS and invasive mammary carcinoma, for example, are already essentially considered separately. These models will profoundly influence the search for biomarkers and the design of dietary and other prevention strategies.

**Literature Cited**


