Targeted Controls in Clinical Immunohistochemistry

A Useful Approach to Quality Assurance

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Few would argue with the premise that the tasks faced by laboratory physicians in the year 2001 are significantly different than they were even 10 years ago. Through the 1980s, for example, the expected role of the surgical pathologist was to establish a timely and accurate tissue diagnosis for any specimen sent to him or her, and providing answers to clinical questions on patient management generally was a straightforward derivative of that activity. Following the advent of the “molecular era” roughly a decade ago, that situation has begun to change. The gene-product “fingerprints” of various microorganisms and neoplasms have now been targeted as points of interest for adjunctive diagnosis and also as possible indicators of prognosis or response to treatment interventions. In particular, proteins that reflect the deletion, mutation, or amplification of germline gene configurations and that also seem to have an active role in cellular transformation or growth regulation command particular attention as “disease modifiers.” In other words, one may hypothesize that interventions that modify or interrupt the intracellular roles of such mediators may influence the disease in question and, in so doing, have a beneficial effect on the organism with that disorder. Aside from representing an advance in therapeutics, this line of reasoning also has had a definite impact on laboratory professionals. We are now expected to abet the assignment of patients to one treatment group or another, based on the molecular and kinetic alterations in diseased tissue that we are given to study. That task has become more and more focused, as therapeutic agents with increasingly greater putative specificity continue to emerge. However, there are several important issues that have received short shrift in our natural enthusiasm to participate in the cutting edge. It is those topics that will serve as the subject of this commentary, which is attached to important observations by Rhodes et al1 in this issue of the Journal.

At this point, virtually all anatomic pathologists are aware of the guidelines that have been established by the US Food and Drug Administration (FDA) in reference to tissue analyses for c-erb-b2/HER-2/neu gene expression by invasive breast carcinomas.2-4 Reagents for both fluorescence in situ hybridization and immunohistochemical analysis have been approved by the FDA to assess the possible amplification of HER-2; these are direct and indirect markers, respectively.5 For immunohistologic studies, a limited number of commercial firms have thus far been FDA certified to market HER-2–related test kits that are applicable to tissue sections. These assume that “2+” (“moderate”) and “3+” (“strong”) immunolabeling of the cell membranes in invasive breast carcinomas correlates with true gene amplification and, therefore, with potential susceptibility to trastuzumab (Herceptin), the therapeutic monoclonal antibody that has been raised against the HER-2 receptor.6-8 Moreover, the manufacturers of such kits have attempted to standardize results by including positive control sections obtained from breast cancer cell lines that are known to show various levels of HER-2 amplification. Nevertheless, as summarized accurately by Rhodes and colleagues,1 only a small number of control sections are included per kit, and, in the interest of cost saving, that limitation (as well as others) has induced enterprising pathologists to deviate from prescribed guidelines for kit use, even at this relatively early stage in the history of trastuzumab administration. In many laboratories, sections of randomly selected breast carcinomas often are being substituted for the specified positive control slides, simply based on their previous subjective
immunoreactivity in the context of clinical testing. However, there often is no attempt to provide stratified controls with $0$, $1+$, $2+$, and $3+$ levels of HER-2 labeling in these “in-house” activities.

Inasmuch as standard cell lines are available in the public domain from institutions such as the American Type Culture Collection, the European Collection for Cell Cultures, and others, there are now readily available, affordable, and well-characterized control samples for immunohistologic and molecular studies that deal with human malignant neoplasms. Four such cell lines (MDA-MB-453, BT-20, MCF-7, and SKOV-3) were used in the study by Rhodes et al., allowing them to reproducibly “titrate” the results of HER-2 immunolabeling to the documented levels of target protein in each of those substrates.

This innovative study provides an easy and comparatively inexpensive method to control an immunohistochemically mediated analysis semiquantitatively. That step, in turn, has several desirable ramifications. First, it allows day-to-day variation in technique in any given laboratory to be identified and, hopefully, remedied. Second, provided that HER-2 antibodies that are not marketed as part of kits have been shown to demonstrate sufficient specificity, they can justifiably be used in a clinical setting if they are titrated against appropriate cell lines. That would further decrease the cost of the analysis and, concomitantly, could well induce the FDA to broaden its certification of additional anti–HER-2 reagents. Third, the use of predefined and widely available control samples for immunohistologic studies sets the stage for a long-anticipated goal: that of interlaboratory reproducibility and comparability in staining results. As stressed in earlier communications, the time to focus attention on quality assurance in immunohistochemistry is upon us. If we do not respond to that challenge and do not use methods such as described in this issue of the *Journal*, less desirable regulatory approaches could well be imposed on pathologists from outside our specialty group.

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References