EDITORIAL

Pre-analytic variables and phospho-specific antibodies: the Achilles heel of immunohistochemistry

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See related research by Pinhel *et al.*, http://breast-cancer-research.com/content/12/5/R76

Abstract

Immunohistochemistry is the most common method for companion diagnostic testing in breast cancer. The readings for estrogen receptor, progesterone receptor, and Her2 directly affect prescription of critical therapies. However, immunohistochemistry is highly sensitive to innumerable pre-analytic variables that result in loss of signal in these assays. Perhaps the most significant pre-analytic variable is cold ischemic time. The work of Pinhel and colleagues in the previous issue of *Breast Cancer Research* examines the effects of cold ischemic time and finds a chilling result. The authors show that while the classic markers may be only mildly affected, phospho-specific markers are highly sensitive to this artifact. As a result, it is likely that future companion diagnostic tests that include phospho-specific epitopes will be reliably done only in core needle biopsies that minimize ischemic time.

Immunohistochemistry (IHC) has been broadly accepted as a companion diagnostic method to assist clinicians in the prescription of targeted therapies. However, it was recently observed that the quality of the biomarker study is highly dependent on quality of the tissue. The quality of the tissue is dramatically affected by pre-analytic variables. Thousands of uncontrolled variables that affect every tissue specimen could alter the results of companion diagnostic testing. Some common examples include cold ischemic time, intraoperative hypoxia, section thickness, type of fixative, processor protocols, and scores of other subtle and not so subtle variables. Although it is impossible to control for all pre-analytic

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variables, efforts to characterize their effects are under way.

In the previous issue of *Breast Cancer Research*, Pinhel and colleagues [1] examined the effects of time to fixation by the measurement of protein expression of traditional breast cancer biomarkers in a timed series of core needle biopsies and the conventional resection specimens. The authors compared the expression of estrogen receptor (ER), Her2, progesterone receptor (PgR), ki-67, p-Akt, and p-Erk in each set and used conventional IHC with semi-quantitative readouts and found no significant difference between the expression of ER, Her2, PgR, and ki-67 in the core needle specimens with a median time difference of 30 minutes. Comparison with tumor resection samples from the same patient also showed no significant difference except that a lower ER value was seen in the conventional resection compared with the biopsy. These findings suggest that if epitope degradation occurs during the ischemic time prior to fixation, its impact may be limited to borderline cases.

The impact for phospho-epitopes is not so subtle. Here, Pinhel and colleagues show a dramatic loss of antigenicity for both p-Akt and p-Erk1/2 when comparing the cores with conventional resections. Although these markers are not routinely used in current diagnostic testing, their potential for sensing pathway activation has made them extremely popular candidate markers for companion diagnostics for kinase inhibitors [2]. The work of Pinhel and colleagues shows significantly lower levels of the phospho-epitopes in tumor resection samples with longer time to fixation. This work suggests that the timing and tissue handling are critical for biomarker assessment of phospho-proteins in clinical specimens. Since delayed time to fixation can alter the phosphorylation status in resection specimens, use of these epitopes in companion diagnostic tests will likely have to be limited to core needle biopsies.

The work of Pinhel and colleagues validates data seen in other studies that have shown the decrease in phospho-protein biomarker expression as a function of

time to fixation [3-5]. Work in our lab has also quantified this loss for these and other phospho-epitopes while showing less change in the non-phospho-sensitive epitopes of the same proteins (Yalai Bai and colleagues, Department of Pathology, Yale University School of Medicine, New Haven, CT, USA, manuscript submitted). Thus, although phospho-proteins are a tempting target, they present unique challenges. Most likely, this is explained by the fact that phospho-epitopes are highly sensitive to phosphatases. In fact, it is the transient balance between kinases and phosphatases that is critical in cell proliferation, cell migration, and other pathways in tumor progression [6]. Most likely, the loss of the phospho-epitopes is due to the unregulated phosphatase activity seen in ischemic conditions or early-stage tissue degradation that occurs prior to fixation.

Can anything be done to address this issue of pre-analytic variability? Efforts have been made to standardize tissue management in the American Society of Clinical Oncology/College of American Pathologists guidelines for Her2 [7] and for ER and PgR [8]. The guidelines stipulate a maximum of 1 hour between resection and fixation and a minimum of 6 hours in fixative. Clinical trial groups have also set guidelines for specimen handling [9]. However, the concept of biospecimen science is still relatively new. Historically, work characterizing time to fixation and other pre-analytic variables was not recognized as important and was difficult to publish. As a result, the issued guidelines are based on a very limited body of literature [10-12]. The paper of Pinhel and colleagues and similar studies are welcome additions to the literature. No doubt, there will soon be others since the National Institutes of Health has recently established the Office of Biorepositories and Biospecimen Research and has funded a series of studies on biospecimen science. The work of the funded investigators and works like this article by Pinhel and colleagues are likely to be cited as key evidence to form the basis of future guidelines for companion diagnostic tests.

Abbreviations
ER, estrogen receptor; IHC, immunohistochemistry; PgR, progesterone receptor.

Competing interests
DLR is a co-founder of, consultant to, and stockholder in HistoRx Inc. (Branford, CT, USA) and Metamark Genetics Inc. (Cambridge, MA, USA). However, this editorial does not refer to any technologies related to those companies. SS declares that he has no competing interests.

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