LETTER TO THE EDITOR

ASSESSMENT OF p53 OVEREXPRESSION BY NON-IMMUNOHISTOCHEMICAL METHODS

The editorials by Wynford-Thomas and Hall and Lane address some very important questions concerning the use of immunohistochemistry for the assessment of p53 protein in tumours. The accompanying papers by Baas et al. and Lambkin et al. further confirm that in order to obtain meaningful results with immunohistochemistry, experimental conditions such as variations in fixation, antibodies, and section pretreatment must be carefully standardized. Moreover, the interpretation of results could be subjective as only a percentage of cells stain positive for p53 in many cases. These and other shortcomings of immunohistochemical techniques have been adequately addressed in the latest editorial.

We agree with Baas et al. that the analysis of p53 mutations at the molecular level is cumbersome, time-consuming, and generally not suitable for routine use at present. Molecular analysis, although often considered the gold standard in terms of its ability to reveal genetic, and presumably protein level, alterations, has its limitations as well, one point being that not all mutations exist in the commonly examined exons 5–9 and some occur in intrinsic regions. Recently, two large studies have established that p53 protein accumulation is an independent risk factor in breast carcinoma and some other studies have presented evidence that p53 gene mutations may be prognostic of unfavourable outcome in other cancers as well.

Not mentioned in the recent editorial, however, were methods other than immunohistochemistry for the detection of p53 protein overexpression. A number of groups, including ours, have developed or used enzyme-linked immunosorbent assays (ELISAs) for p53 protein which are based on non-competitive immunoassay principles employing monoclonal and polyclonal anti-p53 antibodies. ELISAs have a number of distinct advantages over immunohistochemistry. In these procedures, in order to obtain signal, p53 must bind to two different antibodies instead of the single p53-specific antibody used in immunohistochemical methods, with increased antigen specificity being the expected pay-off. Moreover, endogenous peroxidase activity or biotin-binding proteins, which are sometimes present in tissues, are not a source of interference in ELISA assays due to the effective immunopurification of the antigen of interest from all other tissue components. Tissue fixation variability and pretreatment effects could also be eliminated.

From an interpretative point of view, quantitative ELISAs are more objective because the results can be evaluated using numerical cut-off values, simplifying the statistical analysis of both sample data and quality control. ELISA sensitivity is excellent and femtomole to attomole levels of analyte could be routinely measured. The extraction of cellular proteins from tumour tissue is technically simple, arguably requiring less expertise and specialized equipment than tissue sectioning. ELISAs can also be mechanized and throughputs of 100–200 samples per day could be accomplished with minimal effort. Our group and Vojtesek et al. have recently shown that p53 can be assayed in the same tumour extracts prepared for steroid hormone receptor quantification. If p53 is introduced as a routine prognostic indicator for breast cancer patients, its measurement in the same cytosolic extracts used for receptor analyses will save both labour and tumour tissue. Admittedly, one of the current limitations of p53 ELISAs is the lack of an accepted standard preparation for calibration purposes.

In our opinion, if p53 becomes the routine breast cancer prognostic indicator that a number of studies suggest, the ELISA-type assays, similar to those currently used for steroid hormone receptors, should be seriously considered for some of the advantages cited above. We propose that, in addition to immunohistochemistry, ELISA-based
methods of p53 protein detection should be used in future clinical trials examining p53 as a predictor of patient outcome in breast carcinoma.


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author’s reply

Diamandis and Levesque draw attention to the potential value of ELISA assay as a clinical diagnostic tool for the assessment of aberrant p53 expression in breast cancer. The authors correctly point out the desirability of having a quantitative measurement of protein content which would be more objective than immunocytochemistry (ICC). Their arguments could, however, have been more balanced.

For example, one of the advantages that they claim for ELISA is its avoidance of the major uncertainties which result from tissue fixation. While this is of course true, it is the use of frozen tissue which is important, not the technique of analysis—immunocytochemistry is equally free of this problem on frozen tissue! More importantly, they fail to mention a major drawback of ELISA compared to ICC, which is its inability to take account of variations in the proportion of malignant cells present in the sample, which, according to the Vojtesek study,1 can vary at least three-fold. This is, of course, simply a new example of the longstanding dispute between immunocytochemical and biochemical assessment, well illustrated by the problem of oestrogen receptor analysis in breast cancer.

There is no doubt that accurate quantitation of a cellular protein in clinical samples will always be problematic. Ultimately, most clinicians will want a technique which will identity cases having a level of p53 expression associated with poor prognosis. The accuracy required of any routine method will
depend, therefore, on the degree of overlap or separation between this sub-set and the ‘normal’ range. Classical immunocytochemistry (without antigen retrieval) may turn out to be an effective way of making this discrimination. Alternatively, the best compromise may be to use ELISA as the primary measurement, but to control for cellularity as far as possible by visual assessment of parallel histological sections. However, until we know more from research studies about the range and significance of p53 expression levels in breast pathology, it is probably premature to make any firm recommendations for methods of routine clinical assessment.

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