EDITORIAL

Prognostic Significance of p53 Overexpression in Node-Negative Breast Carcinoma: Preliminary Studies Support Cautious Optimism

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In 1993, breast cancer will likely be diagnosed in more than 150,000 women in the United States. In that group, those with axillary lymph node-negative (ANN) disease present one of the greatest dilemmas facing oncologists today. In this issue of the Journal, an article by Allred et al. (1) exemplifies an important trend in the search for a resolution to this problem—the use of this type of prognostic marker. It also highlights some of the problems encountered in such large and inherently difficult studies. This study is also notable because it is one of the last works from an important leader in this field, Dr. William L. McGuire.

The late Dr. McGuire, senior author of the investigation of p53 overexpression in ANN patients, has contributed greatly to our understanding of breast cancer biology. In his numerous reviews and scientific contributions, the target was often early-stage breast cancer. As he stated, "The heart of the matter is: How well do current and proposed prognostic factors separate ANN patients into low- and high-risk groups? Can the results of prognostic factor assays be assembled in an intelligent way to help us to reach the critical decision of whether to use adjuvant therapy or merely observe a particular ANN breast cancer patient?" (2).

Dr. McGuire's research has demonstrated the importance of studying multiple factors on a single tumor and multifactorial statistical analysis of a large patient database to provide "a framework" from which important biologic and prognostic factors could be used to make treatment decisions. He was often an advocate for patients in his clinical approach and cautioned physicians, "In the final analysis, the decision regarding adjuvant therapy for ANN breast cancer rests with the patient. It is the responsibility of the practicing oncologist to help the patient evaluate her prognostic factors, arrive at an understanding of her particular risk of recurrence, and, within this context, weigh the potential benefits and risks of adjuvant therapy." (2).

Prognostic markers that are useful in predicting outcome in breast cancer patients and in identifying those that have a higher probability of deriving benefit from adjuvant therapy have been disappointingly rare. The study by Allred et al. (1) is a commendable investigation of tumor suppressor gene p53 overexpression in tumors from a large cohort (700) of ANN patients. Using relatively common methods including immunohistochemistry or flow cytometry, Allred et al. performed multifactorial analyses to determine the prognostic significance of p53 protein expression, tumor size, estrogen and progesterone receptors, age, tumor cell ploidy, percentage of cells in the S phase (%S phase), and Ki-67 labeling index. Overexpression of p53 protein (secondary to protein stabilization most often associated with gene mutation) was the strongest independent predictor of early disease recurrence, followed by large tumor size and high %S phase. Tumor grade, histologic tumor type, and other prognostic markers were not included in the multivariate model.

The study by Allred et al. (1) confirms the prognostic significance of p53 overexpression in ANN patients reported by two other independent groups of investigators (3,4), who used fewer patients but had longer median follow-up. These findings underscore the need for standardization of p53 overexpression assays, because although the conclusions are generally similar, the methods and tissue samples used were distinctly different. Allred et al. (1) utilized frozen pellets of particulate cell preparations made from breast cancers. Therefore, histologic examination and quantitation of contaminating benign epithelial, stromal, or inflammatory cellular elements in the study samples could not be performed. The p53 assay used a cocktail of antibodies, PAb1801 and PAb240, as well as a scoring system not previously reported. Direct comparison of these methods with previously published assay techniques was not possible. However, in order to compare the immunohistochemical data with findings of genetic alterations of p53, single-strand conformation polymorphism (SSCP) was performed on a subset of tumors. The median clinical follow-up was 54 months.

Like investigators in many other studies (5,6), Allred et al. (1) found some discordance between immunohistochemical data and the results of molecular genetic (SSCP) analysis of p53. On one hand, this discordance could be explained by a lack of sensitivity of the SSCP analysis, a commonly reported problem that has been addressed by others (7,8). This discordance may also be secondary to what the authors describe as "low tumor cellularity" among some samples. A high fraction (50% or more) of contaminating nonmalignant cells can lead to a false-negative finding by SSCP analysis. Accumulation (overexpression) of p53 protein may also result from interactions with other proteins known to form complexes with the p53 protein in vivo (9). Furthermore, tumor heterogeneity and sampling error likely contribute to these discrepancies. Our experience in ovarian carcinoma suggests that there is a strong association between molecular

*See "Notes" section following "References."
genetic and immunohistochemical data (with the use of the monoclonal antibody PAb1801 on frozen or fixed tissues) if molecular genetic analysis of the entire p53 gene is carried out (Kupryjaniczky J et al.: manuscript submitted for publication). We have detected almost all missense mutations (amino acid substitutions) by both methods. In contrast, nonsense mutations resulting in a premature stop codon occurred at random sites throughout the gene and were not detected by immunohistochemistry. Hence, it is likely that immunohistochemical assays detect the majority of tumors with p53 mutations, although this technique clearly has some limitations. It is unclear whether these limitations are significant in a prognostic sense.

In a 1990 National Institutes of Health Consensus Development Conference on the Treatment of Early-Stage Breast Cancer (10,11) characteristics of a useful prognostic factor for breast carcinoma were defined: 1) significant and independent predictive value that has been validated by clinical testing; 2) determination that is feasible, reproducible, and widely available with quality control; and 3) readily interpretable by the clinician, with therapeutic implications. Prognostic factors that were specifically addressed by the panel of experts and came closest to meeting these criteria included tumor size, estrogen and progesterone receptor status, nuclear grade, histologic tumor type, and proliferative rate. Accumulation of p53 protein does not yet fulfill these criteria because a consensus has not yet been reached on methodology, interpretive factors, or quality control issues. Additional studies should now be performed at multiple institutions with standardized methods and patient populations controlled for therapeutic intervention, to validate the clinical importance of these findings. Ideally, large randomized trials that include analysis for p53 protein should also be performed.

We have all seen the headlines and sound bites touting the latest and greatest prognostic marker in breast cancer (based on studies of relatively few or clinically selected patients) and have been sorely disappointed and disillusioned when, a year or two later, these findings could not be confirmed or were less clinically significant than initially implied. Guidelines for study design, reporting, and ethical considerations do not currently exist, although the National Cancer Institute has recently convened working groups to deal with these issues. In addition, the Food and Drug Administration has drawn up a draft list of monoclonal antibodies given priority and temporary exemption in a compliance policy guide that addresses the commercialization of unapproved in vitro diagnostic tests. This list is designed to protect consumers from inappropriate testing practices and currently includes 61 monoclonal antibodies considered to be the standard of care for diagnosis or monitoring of serious disease conditions (12). None of the oncogene markers reported to have prognostic significance in breast cancer have been given this exemption to date; hence, each will have to be labeled either "for investigational use only" or "for research use only." Cautious optimism about p53 overexpression as an extremely important marker of poor prognosis in node-negative breast cancers now seems appropriate. The academic and patient communities that stand to gain from these investigations should insist upon methodologic consensus, proper controls, data verification, and ethical practices in data presentation, publicity, and publication to ensure the greatest benefit from these important investigations.

Much has been learned from past mistakes in prognostic marker study design, statistical analysis, overinterpretation of data, and clinical integration that was premature. Studies on tumors from early-stage breast cancer patients are especially difficult because the disease, patient factors, therapeutic intervention, and observed outcome are so heterogeneous. Substantial numbers of patients are required to demonstrate statistical significance for a given marker. Despite these difficulties, one in nine women will face breast cancer at some time in life, and treatment options will be based on clinical and histologic features of known prognostic significance. Future investigations of this kind surely owe gratitude to Dr. McGuire for his foresight and pioneering efforts.

References

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Notes

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