Expression of the nm23 Gene and Breast Cancer Prognosis

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Carcinoma of the breast is the most common malignancy in women in the United States, and is second only to lung cancer as a cause of cancer-related death in women (1). It has been estimated that 1.5 million women will be diagnosed with breast cancer in the 1990s, and of these 30% will succumb to the disease.

The modalities for the treatment of this disease as well as the aggressiveness of the treatment plan are variable and the decision as to which approach to take can frequently depend on information regarding the nature of the biologic behavior of an individual tumor. As a result, a number of prognostic indicators have been examined for their ability to predict the behavior of a given tumor. The most generally accepted, and to date, best prognostic indicator in the management of breast cancer is the presence or absence of malignant cells in the axillary lymph nodes (2). Patients with involved lymph nodes are known to be at high risk for recurrence and to have a shortened survival; the greater the number of involved nodes, the greater the risk. There is little debate that these patients should receive adjuvant chemotherapy and/or endocrine therapy. A significant number of women diagnosed in 1990, however, had node-negative breast cancer. While these patients as a group have a more favorable overall prognosis than patients with node-positive disease, it is known that between 20% and 30% will experience a recurrence. Given these statistics and the difficulty of effectively treating recurrent disease with curative results, many oncologists have advocated the use of adjuvant therapy in node-negative patients with the intent of benefiting those who might otherwise have a recurrence. Data from several large clinical trials indicate that such a therapeutic approach decreases disease-free recurrence rates in these patients (3-6), and as a result, many believe that adjuvant therapy will improve overall survival in this group of women. In fact, this concept has led the NCI to issue a clinical alert asking physicians to consider the use of this treatment modality in node-negative patients (7). Counter-arguments to the use of adjuvant therapy in all node-negative patients, however, are the short-term and potentially long-term risks of such treatment. Should the 70% to 80% of node-negative patients who will not experience a recurrence be put at risk to benefit those who will? One of the major objectives in breast cancer research over the past decade has been to identify additional prognostic factors that will distinguish those patients more likely to have recurrent and/or aggressive disease. To this end, a variety of potential prognostic factors have been investigated, including tumor size (8,9), histologic and nuclear grades (10), hormone receptor status (11,12), proliferative rate (13-15), growth-factor receptors such as the protein products of the HER-2/neu (also known as the ERBB2) and the epidermal growth-factor receptor (EGFR) genes (16-19), and cathepsin D production (20). While a substantial research database exists for some of these factors, only those node-negative patients with tumors less than 1 cm in size, who are expected to experience recurrences at a rate of less than 10% over a period of 10 years, have been advised against systemic therapy by the most recent NIH Consensus Development Conference (21).

Several reasons exist for the lack of significant progress in first identifying and then implementing the use of new and effective prognostic indicators. Examples of these problems are apparent in the research conducted in several laboratories, including our own, on the utility of the HER-2/neu gene for predicting outcome in human breast cancer. Disagreement concerning what constitutes and how best to measure a significant genetic alteration such as amplification is one such problem (22). Second, both the techniques and the reagents used to measure HER-2/neu expression have been quite variable from...
lab to lab and, as a result, have led to conflicting reports (16). Third, many studies have used inadequate numbers of cases or a clinical follow-up period of insufficient duration to be able to draw a definite conclusion regarding the significance of this prognostic factor (16,22,23). Similar problems have occurred in evaluating almost all of the newer prognostic factors currently being investigated and/or being proposed in the literature.

The report by Hennessy et al, published in this volume of the Journal (24), provides interesting new data on the utility of the molecular analysis of nm23 gene expression and breast cancer prognosis. The nm23 gene was initially discovered by Dr. Patricia Steeg of the National Cancer Institute (25), by use of the technique of differential colony hybridization with related cell lines possessing low and high metastatic properties. This approach was taken in an effort to identify any genes associated with metastatic potential. nm23 mRNA levels were found to be approximately 10-fold higher in two K-1735 murine melanoma lines with low metastatic potential compared with five highly metastatic K-1735 melanoma cell lines. In addition, reduced nm23 mRNA expression was reported by Steeg et al in highly metastatic tumor cells from several other rodent metastasis model systems including N-nitrosomethylurea-induced mammary tumors and HRAS adenovirus 2 E1a-transfected rat embryo fibroblasts (26). Subsequently, nm23 mRNA levels were measured in a small panel of human breast carcinomas using both Northern blot and in situ hybridization techniques (27). The in situ technique was used to specifically evaluate expression in tumor cells compared with normal stromal cells and lymphocytes in the same specimen. Most of the tumors from patients with involved lymph nodes were low in nm23 mRNA content, while adenomas and breast carcinomas from node-negative patients exhibited higher nm23 mRNA levels. These data indicated that increased tumor metastatic potential in at least one type of human tumor was associated with reduced nm23 mRNA levels, and suggested further analysis of the prognostic potential of the nm23 gene in breast cancer.

In the current report, Hennessy et al (24) quantitate nm23 mRNA levels in a breast carcinoma cohort, and correlate nm23 gene expression with histopathologic grade and clinical course. Northern blot analysis was used to measure nm23 mRNA content, which was then quantitated by densitometric comparison to a HeLa cell RNA standard. Using this approach, nm23 mRNA levels varied from 0.1 to 12 units. Tumors were then separated into subsets of low and high nm23 mRNA content based on an arbitrary cutoff value of 2.4 units, yielding 31 high- and 30 low-expressing cases. Significant correlations were observed between nm23 mRNA levels and negative versus positive lymph node status, as well as between nm23 mRNA levels and histopathologic grade (Stages 1 or 2 versus 3, and Bloom and Richardson's grade, respectively). Moreover, patients with high levels of nm23 mRNA expression in their tumors exhibited significantly longer disease-free (P<.002) and overall (P<.003) survival than did patients with low levels of nm23 mRNA expression in their tumors. Twenty six of the tumors in this study were from lymph node-negative patients, and nm23 mRNA levels in these tumors also varied. The authors performed statistical analyses to determine whether expression in this critical subset was associated with clinical outcome and found that patients with high nm23 expression in their tumors exhibited significantly longer disease-free (P<.02) and overall (P<.005) survival. These data, taken together with those of Steeg et al (27), provide clear evidence of an association between nm23 mRNA levels and clinical prognosis in the breast cancer studies done to date.

Further prognostic studies on larger cohorts will be required to confirm the statistical differences in survival within the critical node-negative subset, as well as in node-positive patients. Furthermore, multivariate analyses will be required to determine whether nm23 is a significant independent prognostic variable. Given some of the previously mentioned pitfalls encountered in the measurement of other genes and gene products in human tumors, it is anticipated that additional studies will be needed to carefully and comprehensively study this gene and its expression in human breast cancer. Such studies will serve to validate both the methods of analysis as well as the reagents used; they have been useful in clarifying some of the conflicting data found with other prognostic factors (16). Rosengard et al have prepared affinity-purified polyclonal antibodies to synthetic nm23 peptides, based on nm23 DNA sequence data, and identified a 17 kd nm23 protein in Western blot analysis of lysates of murine and human tumor cells (28). Immunohistochemical analysis using immunoperoxidase techniques to measure nm23 protein levels should offer the advantage of requiring only a tumor section, as opposed to the 0.5-gram amount of tumor used for RNA extraction in the current study, and is another feasible approach to determine relative levels of nm23 protein expression in tumor specimens. Finally, studies in which the prognostic potential of two or more potential factors, such as nm23 expression, tumor size, HER-2/neu and EGFR overexpression, and cathepsin D production, are determined in a single cohort, may be of additional value to understanding and utilizing this gene.

Although the data provide a convincing correlation of nm23 mRNA levels with patient survival, several questions remain concerning nm23 gene expression. The first concerns the specificity of antibody quantitation of nm23 protein levels. Two distinct human nm23 genes have been identified (29) which both encode proteins of approximately 17 kd that are approximately 90% identical at the amino acid level, and Northern blot hybridization data indicate that the nm23-H1 and nm23-H2 genes are independently regulated (P. Steeg, personal communication). Based on their predicted amino acid sequences, it is likely that current antibodies to nm23 synthetic peptides recognize both gene products, and that an antibody specific to one of these peptides may be difficult to produce. Another interesting research question regards the regulation of nm23 gene expression in tumor cells. Preliminary studies in our laboratory suggest an interaction between HER-2/neu and nm33 expression, in that human breast and ovarian carcinoma cell lines transfected with HER-2/neu exhibit reduced nm23 RNA levels compared with mock transfected controls (P. Steeg, V. Campbell, and D. Slamon, unpublished data).

It is unlikely that the analysis of any one gene will satisfactorily explain any or all of the important characteristics of the biologic behavior of a human malignancy. It is more likely that the malignant phenotype results from the interaction of a num-
ber of different gene products and that their presence or absence plays a role in this context. The reduced expression of the nm23 gene in highly metastatic tumor cells has led Steeg et al to postulate that nm23 may function as a suppressor gene for tumor metastasis and thus play a role in the pathways that govern this important pathophysiological event. Transfection studies are underway in her laboratory to answer this question (P. Steeg, personal communication).

References