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PERSPECTIVE

Gene-Expression Profile: The Future in the Outcome Prediction and Treatment of Breast Cancer

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ABSTRACT

The malfunctioning of specific genes drives probably the development and progression of neoplastic disease. Indeed, recent studies using microarray analysis provide evidence that breast cancer tumors can be classified according to their gene-expression profiling into subgroups with poor prognosis and good prognosis. These works represent a considerable advance in the clinical application of microarray technology.

The fascinating of these studies is that the DNA-microarray data predict the outcome –overall survival and distant metastases- much better than such classic prognostic indicators as nodal status, grade, stage, and estrogen-receptor status.

The finding that the genetic signature of a breast cancer is strongly associated with outcome independently of nodal status has important biologic implications and may change the current therapeutic strategy regarding clinical decision making about adjuvant chemotherapy in some individuals who at present either overtreated or undertreated.

Although the incidence of breast cancer in the western world still remains high, mortality has been reduced over the last decades. This improvement is partially attributable to an effective postoperative adjuvant treatment -radiation, chemotherapy, hormone therapy.[1-5] Decision making is based on a combination of clinical and tumor characteristics: age, tumor size, lymph-node and estrogen-receptor status, and histology (type, grade).[3-5] However, the ability of these criteria is imperfect and as result some patients undertreated and others overtreated with respect to adjuvant treatment.[6] So some individuals more harm than benefit from such a conventional risk prediction-based adjuvant treatment. For example, about one third of women among those with node-negative breast cancer will develop recurrence[1,2] and might benefit from an adjuvant systemic therapy, and about one third among those with node-positive disease will remain free of recurrence 10 years after treatment[1,2] and thus would eventually benefit avoiding a toxic adjuvant chemotherapy.

Why do some individuals with identical clinical, histological, and therapeutic criteria recur after treatment and die of the disease, while others not? It is apparent that still unidentified molecular factors should determine the ability of the primary tumor to recur or metastasize. Conventional staging systems currently used have limited predictive power for an individual patient. The rational for the search of new molecular markers is reliably receiving increasingly considerable attention because this is the most promising way towards a highly accurate outcome prediction. Predicting who individual is really at very high or low risk for metastasis we can best design an adjuvant treatment highly effective targeting to an individual patient resulting in substantial benefits in both survival and quality of life.

Molecular analysis of the primary tumor raises strong hope towards an accurate prediction of tumor behavior and represents the current major focus of cancer research. However, until now thousands of studies have failed to identify new factors with predictive power,[7] with the exception of estrogen and progesterone receptors and the *HER/neu* gene that are the only molecular targets routinely used in breast cancer.

The Human Genome Project revealing detailed information about the structure of all human genes, makes now feasible the search for such predictive markers. Powerful new high-performance screening techniques have been developed for molecular analysis. One of these new methods is the DNA microarray, which allows simultaneous analysis of the expression of thousands of genes in a tissue in a single experiment. The

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different kinds of tumors.

Microarrays are small glass plates or nylon membranes to which specific sequences of thousands of genes adhere. Two different kinds of target DNA sequences are widely used: complementary DNA (cDNA) sequences or oligonucleotide sequences representing small but highly specific segments of the target genes. As probes, complementary DNA or complementary RNA (cRNA) prepared from RNA derived from tumor tissue and the appropriate control tissue are used. There are two main ways of using data from a DNA microarray: systematic search for single genes that may associated with prognosis and the use of the entire set of expressed genes to classify tumors according to the microarray derived gene-expression profiles.[8]

Histologically similar tumors can be classified, on the basis of these patterns, into specific subtypes.[9] Using complementary DNA (cDNA) microarrays to analyze breast-cancer tissue, Perou et al. identified two subgroups with distinct gene-expression profiling.[10] These two subgroups had different clinical outcome.[11] In addition, microarray analysis has been used to distinguish breast cancers associated with BRCA1 or BRCA2 mutations[12,13] and to determine estrogen-receptor status[10,13,14] and lymph-node status.[15,16]

Recently, van 't Veer et al. identified a specific gene-expression profile that is associated with prognosis in patients with breast cancer.[13] Using oligonucleotide microarrays the expression patterns of 98 women younger than 55 years with primary tumors less than 5 cm in diameter and with lymph-node–negative disease were analyzed. Within a set of 25,000 genes, they could identify a set of 70 genes only with an expression pattern that allowed highly accurate classification of the patients into those with a poor prognosis and those with a good prognosis.

More recently the same research team reports an extension of their original study that includes not only patients with lymph-node–negative but also with lymph-node–positive breast cancer.[17] The previous established 70-gene prognosis profile[13] was tested in this study. The 295 stage I or II breast cancer patients were classified\on the basis of their expression prognosis profile into two groups; 180 patients had a poor-prognosis signature and 115 patients had a good-prognosis signature.

At 10 years, overall survival and disease-free from distant metastases rates were significantly lower in the poor-prognosis (54% and 50%) than in the good-prognosis signature group (94%, and 85%) respectively. The estimated hazard ratio for distant metastases in the group with a poor-prognosis signature, as compared with the group with the good-prognosis signature, was 5.1 (95% CI, 2.9-9.0; P<0.001). This ratio remained significant when the groups were analyzed according to lymph-node status. The hazard ratio for distant metastases in the group of 151 patients with lymph-node–negative disease and the group of 144 patients with lymph-node–positive disease was 5.5 (95% CI, 2.5 to 12.2; P<0.001) and 4.5 (95% CI, 2.0 to 10.2; P<0.001) respectively among those with a poor-prognosis signature as compared with those with a good-prognosis signature.

These data clearly indicate the prognostic power of gene-expression profiling, but the fascinating result with potential important clinical implication derived from the multivariable analysis. The gene-expression signature was a stronger independent predictor of the outcome than any other currently used prognostic indicators as nodal status, stage, grade, and estrogen-receptor status. The poor-prognosis signature was by far the strongest predictor of the likelihood of distant metastases, with an overall hazard ratio of 4.6 (95% CI, 2.3 to 9.2; P<0.001). The finding that the development of hematogenous distant metastases are independent from the presence or absence of lymph node metastases may suggest that hematogenous spread and lymphatic spread of the disease are events that represent two distinct pathways in the genetic evolution of cancer.[6]

But the most important clinical implication of the findings by van 't Veer et al.[13] and van de Vijver et al.[17] is the ability of the genetic signature to identify patients at high-risk or low risk within the groups with node-negative or node-positive cancer.

The ability of prognosis signature in the study by van de Vijver et al. to predict the risk of distant metastases in node-positive patients is clinically important. Currently all node-positive patients receive adjuvant chemotherapy since the presence of node metastases is by itself a strong predictor of poor survival. The importance of the prognosis profile is that node-positive patients with poor-prognosis profile could eventually benefit from a more aggressive chemotherapy. However, no estimation can be made for the prognostic value of the profile in patients with untreated lymph-node–positive disease, since most of these patients in this study received adjuvant chemotherapy or hormonal therapy (120 of 144 patients).[17]

Most patients with node-negative disease can be effectively treated with local therapy consisted of surgery and radiation since there is no evidence that cancer cells have spread beyond the primary tumor. However, a proportion of node-negative patients develops recurrence. On the basis of various clinical and histological characteristics, the St. Gallen criteria[4] and the National Institutes of Health (NIH) consensus criteria[5] have been developed to classify node-negative patients into low-risk and high-risk subgroups. Decision about adjuvant treatment is currently based on this classification. van de Vijver et al. compared the predictive value of the gene-expression profile, the St. Gallen and the NIH subgroups. This comparison shows a more accurate classification of node-negative patients by prognosis profile. Since the St. Gallen and the NIH criteria misclassify patients who would be overtreated or undertreated in current clinical practice, the prognosis profile-based classification into low-risk and high-risk subgroups would result in a more effective adjuvant treatment targeting to the appropriate individual patient.

The fact that prognosis profile is based on a small number of genes raises strong hope for the development

of a new highly accurate, affordable, and commercially available biologic staging system. Indeed, the set of genes that distinguished the good-prognosis and poor-prognosis groups consisted of only 70 genes. The majority of genes do not obviously affect the clinical outcome of breast cancer.

The results of the studies using cDNA or cRNA data are just one example of how gene-expression profiling can provide highly useful prognostic information, point out Sauter and Simon,[8] who believe that it is almost certain that analogous studies will yield similar results for virtually all other types of tumors. Moreover, they anticipate that simultaneous expression analysis of the majority of human genes will identify molecular profiles that are linked to the response to treatment.[8]

It is certain that gene-expression profiling will increasingly be received attention. However, there are several limitations and many questions should be answered before molecular profile staging system can be intergraded into the routine clinical management of breast cancer. First of all, the results obtained by these studies[13,17] should be validated by other research teams in larger groups of patients since we have experienced many times over the last years that initially strongly promising data not to be subsequently reproduced in other laboratories.

An eventually validation will open the way for testing the predictive power of molecular profiling for clinical decision-making treatment. Randomized clinical trials are needed for testing the effectiveness of adjuvant systemic therapy on molecular profile-based subgroups of patients. Only the results from such trials can establish whether currently undertreated or overtreated patients may benefit from a prognosis profile-based adjuvant treatment. Furthermore, new studies are needed to evaluate critically whether the analyzed set of 70 predictor genes needs to be refined or expanded[6] to increase the accuracy of predictive power of gene-expression-based staging systems. A major obstacle for broad application of gene-expression profiling and other new techniques will be the requirement of fresh tumor tissue for DNA-microarray analysis,[8] that is in contrast to the established, inexpensive method of formalin-fixed tissue handling in universal use.

The data of the gene-expression profile studies reflect the rational in the incorporation of microarray technology into clinical use. These exciting DNA-microarrays findings provide evidence that the way towards a highly accurate prediction of the outcome in an individual patient has been opened. A genetic signature-based targeted adjuvant conventional treatment in individuals is the next step. Our great expectation in the new millennium however, is the development of new molecular agents targeting genetic abnormalities that drive cancer evolution, progression and metastatic potential of a tumor.

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