

# Gene Expression Patterns Give Valuable Predictions for Breast Cancer

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**There are numerous women who do not react to breast cancer treatment in the same way. So how do you predict which course of treatment will be beneficial? By the use of DNA microarray analysis it is now possible to see patterns in gene expression, and so classify breast carcinomas, in order to deliver a more tailored treatment for each patient. The more accurate prognostication allows a better selection of patients to be more fitted for adjuvant systemic therapy. Adjuvant therapy has a large hand in stopping the recurrence of the cancer as well as the patient's chance of survival. It is therefore important to identify the patterns of an aggressive cancer's genes. There is always room for improvement however, and scientists are still trying to become more and more accurate in their testing, as well as treatment. These treatments may include radiation, new chemotherapy drugs, targeted therapies, bisphosphonates, Denosumab, and vitamin D.**

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## Introduction

One in eight women in the United States will develop breast cancer in their lifetime. With so many people being effected further identification of tumor biology is important. We are able to identify lymph node metastases, histologic grade, expression of steroid and growth factor receptors, estrogen-inducible genes, proto-oncogenes, and mutations in the TP53 gene (2). All of the factors are useful in prognosis however lack individual prognostic implications. The cellular and molecular heterogeneity of breast tumors and the large number of genes involved in controlling cell growth, death and differentiation (2), stress the importance of researching the expression patterns in a multitude of tumors. Those tested were of patients with primary breast carcinomas who had both been disease free after at least 5 years and those who had developed distant metastases, as well as BRCA1 and BRCA2 positive patients (1). BRCA1 positive indicates a mutation in the BRCA1 gene, which is responsible for repairing DNA or destroying cells that cannot be repaired, while the BRCA2 gene is responsible for creating the protein that repairs chromosomal damage.

## Recent Progress

To begin the testing, RNA was isolated from each patient's tumor material and was used to create complementary RNA. By pooling equal amounts of the cRNA from each of the sporadic carcinomas, a reference pool was created (1). Two hybridizations were carried out on each tumor using a fluorescent dye reversal technique on microarrays (1). The genes on the microarrays numbered in the 25,000's and were synthesized by inkjet technology. The scanned images showed the fluorescence intensities, and therefore generated the transcript abundance of a gene in relation to the reference pool created prior. These genes represent inherent properties of the tumors from which they were selected.

With the properties of the tumors determined, an analytical method called significance analysis of microarrays (SAM) was used to search for genes that correlated with patient survival (2). A score from SAM that is negative means the higher expression correlates with longer survival. When the score is positive it indicates the higher expression correlates with shorter survival (2). So each gene's abundance can be connected to the survival rate of the patient, allowing certain genes to be noticed as relating to overall outcome.

Dendrograms also show relatedness of the breast tumors. A dendrogram is a tree diagram, which illustrates clusters of genes. The relatedness of the breast tumors tested can be depicted by the length and subdivision of the dendrogram's branches (1). The dominant results put the gene expressions into two distinct groups. It was shown that only 35% of the patients from the first group were revealed to develop distant metastases within 5 years, while 70% of patients from the second group showed progression in the disease, revealing two types, good prognosis and poor prognosis type tumors (1).

In order to better understand the genes dominantly expressed by the tumors, and better distinguish the two clusters, or groups, the genes were associated with histopathological data. Of the 39 immunohistochemically stained tumors that were Estrogen Receptor (ER) negative, 34 of them belonged to group two of the dendrogram (1). Other dominantly expressed signatures were also related to the ER in group two. A group of down regulated genes was represented containing the ESR1 gene and genes that co-regulate with ER, such as ER target genes. Another dominant gene cluster in group two includes several genes expressed by B and T cells associated with lymphocytic infiltration (1). Of the 18 tumors that were BRCA1 carriers 16 were also found in group two intermingled with sporadic tumors (1). This finding concurs with the knowledge that BRCA1 tumors are ER negative and produce larger amount of lymphocyte infiltration. The gene factors in breast cancer diagnosis can now be seen in relation to one another as well as applied to a grouping system that better determines their survival rate. Group one in the dendrogram consists of tumors of BRCA2 carriers. Concluding, that breast cancer group one and two are different in their ER status and lymphocytic infiltration.

In order to fully identify the intrinsic characteristics of breast tumors and more accurately diagnose on a tumor-to-tumor basis, these two groups can be broken down into subtypes by hierarchical clustering. Group two contains three subgroups, basal-like, ERBB2+ and normal breast-like. The basal-like subtype was depicted by a high expression of keratins 5 and 17, laminin, and fatty acid binding protein 7 (2). Subtype ERBB2+ was characterized by a high expression of several genes in the ERBB2 amplicon at 17q22.24, including ERBB2 and GRB7 (2). In the normal breast-like group the highly expressed genes included ones expressed through adipose tissue and other nonepithelial cells, as well as those of basal epithelial genes, but with a low expression of luminal epithelial genes. Subtypes of group one, the ER+ group, have two, possibly three branches, which include the luminal subtypes A, B, and C. Luminal subtype A portrayed the highest expression of the ER  $\alpha$  gene, GATA binding protein 3, X-box binding protein 1, trefoil factor 3, hepatocyte nuclear

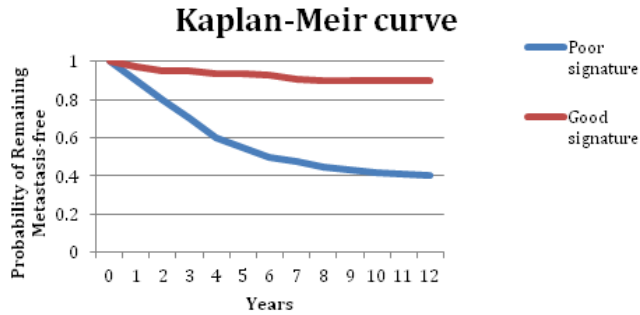
factor 3  $\alpha$ , and estrogen-regulated LIV-1 (2). Luminal subtypes B and C are both luminal-enriched genes and show low to moderate expression of the luminal-specific genes, which include the ER cluster (2). This group could be considered one subtype or separated into B and C. Subtype C is distinguished from B by the high expression of a novel set of genes whose function is yet unknown (2). The use of the subtypes over the two main groups is beneficial in better classifying a patient's tumor as a good or bad prognosis.

With these specific identifications a gene-expression profile of 70 genes that are associated with the risk of early distant metastases can be made (3). In order to turn this prognosis profile into an estimate of their clinical outcome, you can calculate their probability of remaining free of distant metastases. A Kaplan-Meier curve demonstrates the significant difference in the probability of overall survival between poor prognosis tumors and the good prognosis signatures as a ratio 5:1 (fig 1). The overall gene analysis profile is, therefore, a strong independent factor in predicting disease outcomes (3).

## Discussion

The prognosis profile formed is a strong predictor in both lymph-node negative or positive breast cancer and so is very detailed and important in determining whether the tumors are of poor prognosis, and in need of strong treatment or not. This could be beneficial in avoiding over treatment, and putting the patient through more pain than necessary, or under treatment, and allowing the tumor to return. In regards to the past identification of the tumors by the St. Gallen criteria<sup>3</sup>, or the National Institutes of Health (NIH) consensus criteria<sup>4</sup> method, the new method of gene-expression profiling identified what the other methods saw as low-risk patients as having a higher likelihood of metastasis (3). Also, the high-risk patients identified by the new method tended to have a higher rate of distant metastasis than did those identified by the previous methods. We are therefore coming closer to catching aggressive cancers earlier, which is very beneficial as aggressive breast cancer is exceptionally hard to treat. These findings argue against the belief that metastatic potential is acquired later during tumor development, but rather early gene signs can be seen, allowing for early testing. However, it is still unclear if these findings might hinder early treatment as it attempts to hinder a tumors metastatic potential. The classification of patients into good or poor prognostic groups, low-risk or high-risk, is useful in guiding adjuvant or adjuvant systemic therapy. Utilizing these guidelines up to 90% of lymph-node-negative, young breast cancer patients are candidates for adjuvant systemic treatment and may not benefit from adjuvant treatment (1). Now that we have the means to better identify these tumors we will be able

to produce more targeted drugs that can take advantage of these gene changes.



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