

Each patient's cancer is like fingerprint: where are we in molecular profiling in breast cancer diagnosis?

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Abstract

Breast cancer is the leading cause of cancer related deaths in women. Since every patient has unique molecular profile of tumorigenesis, efficient treatment choice should be tailored according to each patient's gene expression profile. Therefore, it is critical to investigate molecular patterns of each patient before choosing the right treatment. For this aim, many commercial breast cancer subtyping methods are available. This review focuses on new developments and methods in molecular profiling of breast cancer.

Keywords: Molecular profiling, breast cancer, tailored therapy

Introduction

Breast cancer is the leading cause of cancer related deaths in women [1]. Traditionally, a thorough evaluation of a breast cancer patient includes determination the dissemination of disease and the assessment of the tumor size, axillary lymph nodes status, histological type, nuclear/histological grade, status of hormone receptor [(estrogen receptor (ER); progesterone receptor (PR)], and Her-2/neu receptors [2-4]. However, tumors with identical histopathology may progress differently, respond to therapy differently, and may result in different disease outcomes. Thus, a new pathological sub-classifications and new molecular diagnostic techniques have been sought in recent years.

By some recent studies it is now well understood that the underlying biological behavior of a tumor reflected in its gene expression is a powerful illustration to define pivotal oncogenic pathways.

Recent trials focusing on gene expression profiling of the tumor indicate that a metastatic risk of a patient is hidden in the gene expression pattern derived from the primary tumor. It should be noted that the metastatic tumor can also be genetically different from the primary tumor, thus a genetic diagnosis of metastatic lesion as well as the primary tumor should also be determined for tailored therapy.

DNA microarray platforms for profiling gene expression in tumors were invented very recently, and breast cancer is the earliest and most intensely studied disease using this technology. The molecular signatures provide diagnostic tools as well as prognostic and predictive gene signatures, and may identify new therapeutic targets. Prospective trials are now underway to determine the value of such tools for clinical decision making in breast cancer.

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Classification of breast tumors based on molecular profiling studies

Breast cancer is a morphologically and genetically heterogeneous disease. Hence, response to treatment as well as the prognosis of two patients with the same stage of breast cancer can be very divergent.

In the very last years, molecular profiling has let us to understand the genotypic characterization of breast cancer and also potentially to discover new molecular biomarkers among cancers with similar histological appearance.

Perou et al. divided the breast cancers into four groups differentiated by expression patterns in several groups of genes; basal-like, Her-2/neu +, normal-breast like, and luminal A and B types [5].

Based on molecular findings, ER status is the most evident classification of breast tumors, since ER status of a tumor has a remarkable impact on the genes expressed by the tumor [5-7]. While the ER positive subtypes included Luminal A and B, the ER negative subtypes included the Her-2/neu+, having expression of Her-2/neu-related genes and basal like subtype with very low expression of Her-2 related genes, but high expression of a group of genes characteristic of normal basal epithelial breast tissue. Luminal subtypes of breast cancer express increased levels of cytokeratin 8 and 18 in addition to those genes associated with ER expression while basal like subtypes of breast cancer express increased levels of cytokeratin 5 and 17 and low levels of ER and genes, whose expression is linked to ER [5, 8, 9]. Similar sub-classification of breast cancer tumors into Luminal and basal like types using different analyses have been done by different investigations [10-12].

From these studies, it is possible to say that all of the luminal groups of breast cancers are ER positive and nearly two thirds of them are of low or intermediate histologic grade, whereas 95% of basal-like cancers are ER negative and most of these tumors

are high grade [13]. Although, most of the (80-90%) triple negative tumors (ER, PR and Her-2/neu negative) similar to the basal like genotype, they are heterogeneous and can be divided into multiple additional subgroups [14, 15]. The basal-like tumors have no ER and Her-2/neu expression and feature more frequent overexpression of basal cytokeratins, epidermal growth factor receptor and c-kit [14]. Unlike Luminal B tumors, Luminal A tumors have the highest ER expression level as well as high expression levels of GATA-binding protein 3, X-box binding protein 1, trefoil factor 3, hepatocyte nuclear factor 3, and LIV-1 [8].

Germline mutations in BRCA1 and BRCA2 genes, which account for most of the hereditary breast cancers, have been shown to be effective on the genes expressed by tumors [7, 16]. Microarray studies have also been used to classify subgroups of these familial breast cancers, which account for 8-10 % of all breast cancer cases [17]. Tumors with BRCA1 and BRCA2 mutations, each display characteristic gene expression profiles. While most of the BRCA1 tumors are basal-like, BRCA2 tumors make up a more heterogeneous group [9, 18, 19].

Currently available microarray tools

In general, microarray technologies based on the manipulation and interpretation of cDNA arrays generated by converting mRNAs isolated from a variety of tissue types to cDNAs, which are then fixed to a solid substrate that allows quantization of these cDNAs by the degree of fluorescence of each probe is quantitated, and represents the abundance of that specific gene transcript, enumerated as either a ratio to a reference sample or as an absolute intensity value. Currently, many commercially available prognostic breast cancer tests based on gene expression technology are available. There are also research based pathway or disease focused

microarray products which haven't been validated on patients are available.

a) Amsterdam 70 gene MammaPrint assay was the first microarray based multigene assay for breast cancer, which was identified by van't Veer and colleagues [7]. This assay includes 70-genes which are mainly focused on proliferation, genes associated with invasion, metastasis, stromal integrity and angiogenesis. The selection of the most optimal gene set to be included in the assay was performed by comparing the gene expression profile of two distinct patient populations that correlated with clinical outcome [20]. This test is currently designed as a pure prognostic assay and is offered as a prognostic test for women under the age of 60 with either ER-positive or ER-negative, lymph node-negative breast cancer and now available as a commercial laboratory test called MammaPrint (Agendia BV, Amsterdam, The Netherlands). The MammaPrint assay is at its best when identifying cases at the extremes of the spectrum of disease to identify of patients with a very good or very poor prognosis. It has not yet been studied if the assay can also predict sensitivity to various treatment modalities. It is important to note that MammaPrint® is also the first FDA-approved in vitro diagnostic assay for patients with node-negative breast cancers [21].

b) The 21 gene Recurrence Score (Oncotype DX™) is a multiplex prognostic and predictive RT-PCR assay which distinguishes good from bad prognosis following adjuvant tamoxifen for patients, using an analysis of the expression of 21 known genes. These genes are mainly associated with proliferation, HER2 and ER signaling. The original 16 cancer related genes with five reference genes that calculate the recurrence score (RS) were discovered on archived paraffin embedded samples by transcriptional profiling and then converted to RT-PCR assay. Oncotype DX determines the 10-year risk for disease recurrence in patients with ER-

positive, lymph node-negative tumors using a continuous variable algorithm and assigning a tripartite RS (≤ 17 , low risk; 18–30, intermediate risk; >30 , high risk) [22].

These two tests mentioned above are the most popular ones that have been used for molecular diagnosis of breast cancer. The other available tests are as follows:

c) The H/I™ (Also known as two-gene expression ratio) is a multiplex RT-PCR-based on the ratio of the relative mRNA expression of the homeobox gene-B13 (HOXB13) and the interleukin-17B receptor gene (IL17BR) to predict recurrence in patients with ER-positive, lymph node-negative primary breast cancer. This test requires formalin -fixed and paraffin-embedded tissue for RT-PCR assay [23].

d) Celera Metastasis Score™ prognostic 14-gene multiplex RT-PCR-based assay is also indicated for ER-positive, lymph node-negative tumors treated with tamoxifen. The Metastasis Score™ for breast cancer predicted a 3.5-fold difference in risk between the 20% of women at the highest risk and the 20% of women at the lowest risk for disease recurrence [24].

e) The Rotterdam 76-gene signature was developed to identify the patients with lymph node negative breast cancer that would benefit from adjuvant therapy, independently of the hormone receptor status [25, 26]. This test has mainly consist of proliferation genes and no genes in common with either oncotype DX™ or MammaPrint™, and run on the Affymetrix U-133 GeneChip™ System (Affymetrix, Inc., Santa Clara, CA). It requires fresh/frozen extracted mRNA and, similar to MammaPrint™, has not been validated for use on paraffin embedded tissues or core biopsies.

f) The invasiveness gene signature (IGS) is a prognostic assay which consists of 186 genes related to tumor stem cells that also use the Affymetrix U-133 GeneChip™ System. This assay is used for both node-

negative and node-positive and both ER-negative and ER-positive patients [27].

g) Breast BioClassifier is a qRT-PCR assay which can identify the different subtypes of breast cancer (luminal-A, luminal-B, Her-2, and basal-like) as a prognostic risk assessment tool [28]. The assay consists of 50 classifier genes and five house-keeping genes are measuring simultaneously, using a 384-well format in the LightCycler 480 system. The Breast BioClassifier can be used for different molecular subtypes of ER-negative/positive breast cancer, and determines the patients may benefit from personalized chemotherapy.

The Human Breast Cancer and Estrogen Receptor Signaling RT² Profiler™ PCR Array (SABiosciences, Frederick, MD, USA) analyses gene expression profiles of 84 genes associated with breast cancer regulation and prognosis, estrogen receptor-dependent signal transduction and response of cancer cells to chemotherapy. Pathway focused DNA microarrays are commercially available. However, these assays have not yet been validated for breast cancer patients. This assay has been studied at our research lab since December, 2008, and we have some preliminary results of Turkish breast cancer patients' genetic signature.

Table 1: mRNA expression levels of genes from Turkish breast cancer patients. Significant changes in mRNA levels of some genes related to breast cancer and estrogen receptor signaling pathway that are grouped according to molecular subtypes in 12 Turkish breast cancer patients (Preliminary findings from Ege University Oncology Research Lab)

Gene	Significant Fold Changes		
	Luminal B (n=7)	Her-2 (+) /ER(-) (n=4)	Basal Like (n=1)
Estrogen Receptor 1 (ESR1)	+11,808	-7,359	-11,437
Keratin 18 (KRT18)	+5,273	+3,011	-8,789
Keratin 19 (KRT19)	+48,946	+7,730	-5,030
Mucin 1 (muc1)	+42,316	+3,581	-5,189
Topoisomerase (DNA) II Alpha (TOP2A)	+12,236	+11,757	-3,483

h) Prediction Analysis of Microarray (PAM50) was designed to determine a risk of recurrence (ROR) score for patients with breast cancer. This test measures the expression of 50 genes to identify the subtypes of breast cancer. It requires formalin-fixed paraffin-embedded tissues for RT PCR method. PAM50 test also provides quantitative determination for proliferation, luminal gene expression, ESR1, PGR, and ERBB2 [29].

Experience of Ege University Medical Oncology research lab on molecular profiling of breast cancer

One of the latest developments of gene expression profiling is a pathway focused PCR Array system which uses SYBR Green-based real-time PCR technology.

The fresh tissue samples were obtained from 12 breast cancer patients operated in General Surgery Department at Ege University. Normal and tumor tissues (24 samples) were taken from the same patient during the operation. Samples were stored in RNA stabilizing solution. RNA was isolated by using RNA purification kit (SABioscience, USA). The Human Breast Cancer and Estrogen Receptor Signaling RT² Profiler™ (84 gene) PCR Array (SABioscience, USA) was used to identify differentially expressed mRNA profiles. The conventional clinicopathological data of the patients were compared with the molecular findings.

According to our preliminary findings, seven of 12 patients were Luminal B subtype, 4 of 12 patients were Her2(+)/ER(-) subtype and one patient was

basal like subtype. mRNA levels of cyto keratin 18 (KRT18), cytokeratin 19 (KRT19), mucine 1 (MUC1) and topoisomerase 2 (TOP2) genes were up regulated in luminal B and Her-2/neu (+)/ER(-) subtypes, however, those genes were down regulated in basal like subtypes. As expected, mRNA levels of estrogen 1 (ESR1) gene was down regulated in both Her-2/neu (+)/ER(-) and basal like subtypes, whereas it was up regulated in luminal B subtypes. A strong correlation was observed between conventional pathological data and pathway related mRNA expression profiles of patients (Table 1).

Conclusion

It is found that breast cancer patients belonging to different subclasses had significantly different outcomes from a survival analysis and prognostic factors based on clinical and histopathological variables [8]. Thus, it is needed to identify more accurate prognostic indicators [8, 9, 22, and 26]. ER protein expression status, histological grade, lymph node status, HER-2/neu gene amplification, p53 mutation status, inflammatory breast cancer, and carcinoma-derived stromal signatures have been defined with molecular profiling studies [2-4, 6-8, 30-32].

The advantage of molecular profiling in cancer is providing of individualized treatment for each patient with different stages of the disease and thus, gaining maximal therapeutic benefit from chemotherapy with minimal toxicity. Thereby, it is possible to deliver the appropriate drug to the right patient, and decreasing the use of other unnecessary drugs. Future investigations in oncology are focused on the individualizing cytotoxic therapy, although similar studies for endocrine and biologic therapy are also going on.

Conflict of Interest

The authors declared that they had no conflicts of interest.

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