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Molecular Fingerprints and Biomarkers of Breast Cancer

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<http://dx.doi.org/10.5772/66899>

Abstract

Substantial progress has been made over the past three decades in understanding breast cancer (BC) molecular biology, genomics, and targeted therapy. The recent comprehensive molecular and pathological diversity observed in BC patients indicates that BC is not a homogeneous disease; It may be appropriately defined as a myriad of diseases. The explosion of molecular information in the past 10 years has led to a better understanding of the biologic diversity of breast cancers (BCs), and clues to the different etiologic pathways to BC development. It will be useful to study the epigenetics of BC cells and define the mechanisms of both genetic and epigenetic driving alterations beside the mutations. Identifying the oncogenes and tumor suppressor genes is the purpose cancer diagnostics and therapeutics. Oncogenes as well as novel ones involved in the significantly altered regions would enable researchers to identify new causes and molecular pathways that may be targeted at BC treatment. Our main goal is to provide comprehensive understanding of underlying molecular mechanisms and hallmarks of BC, focusing on the identification of fingerprints and novel molecular targets that will greatly improve the cancer predictive, prognostic, and diagnostic biomarkers and, in addition, the possible targets for novel therapies.

Keywords: breast cancer, carcinogenesis, molecular markers, omics, personalized medicine

1. Introduction

Cancers, including breast cancer, are generally thought to develop from a single cell in which mutations and/or epigenetic events have modified the function of genes responsible for cellular

growth regulation. These events establish the malignant phenotype, and subsequent molecular events may lead to the emergence of malignant subclones with enhanced growth and metastatic potential [1]. Cancer cells, replicating inappropriately, eventually interfere with normal tissue and organism functions, cause morbidity and may ultimately prove fatal in the absence of effective therapy. The rate of growth of tumours including, breast tumours, is determined by a balance between cell proliferation and cell death; if the rate of proliferation exceeds that of death, tumour growth will occur. Not surprisingly, many of the genes involved in neoplasia turn out to be concerned with control of cell death, as well as with control of cell proliferation [2].

The genes involved in neoplasia are usually classified as oncogenes or tumour suppressor genes, depending on whether the affected gene has gained or lost function in its mutated form. In keeping with this model, breast cancer is the result of imbalance in complex regulatory controls of cellular development and growth. Genetic abnormalities detected in breast carcinomas include mutation and amplification of oncogenes, mutation of tumour suppressor genes, and loss of heterozygosity at certain chromosomal loci. Sex hormones, growth factors, oncogenes, and tumour suppressor genes all regulate gene expression and thereby influence growth and function of breast epithelial cells. Influences favouring cell proliferation or inhibiting cell death may promote tumour progression [3].

Breast carcinoma is phenotypically complex: carcinoma in situ and invasive carcinoma may coexist, as mixed histological types of invasive carcinoma, and infiltrating ductal carcinomas often contain areas with different grades of disease. This morphological heterogeneity mirrors molecular heterogeneity, as well as morphologically similar tumours may differ in genetic and metabolic processes, and specific genetic abnormalities may influence clinical outcome [4, 5].

Prognosis and likely responses to therapy are clinically important in breast cancer and many variables have been evaluated. Classical morphological variables including histological type, tumour size, grade, lymph node status, and whether or not there is blood or lymphatic vascular invasion remain the strongest predictors of tumour behaviour. Attempts to evaluate breast tumour prognosis from individual or combined expression of variables such as oestrogen and progesterone receptor, cell proliferation index, S-phase fraction, DNA ploidy, growth factors and their receptors, oncogenes, tumour suppressor genes, proteases, components of the plasminogen system, and cell cycle regulators have yet to match the clinical utility of the classical morphological factors [6].

The chief forms of carcinoma of the breast are breast cancers that are classified into those that have not penetrated the limiting basement membrane (noninvasive) and those that have (invasive). The WHO current classification [7] is illustrated in **Figure 1**.

Breast cancer is the most commonly diagnosed cancer in women with an increased incidence of 14.1 million new cases in year 2012 and high mortality rate about 8.2 million deaths all over the world [8]. The incidence rate is expected to increase by year 2020 to reach about double the rate in 2012 [9]. Young women aged 20–59 years are expected to suffer from breast cancer with increased rate of death from cancer among their age group [10].

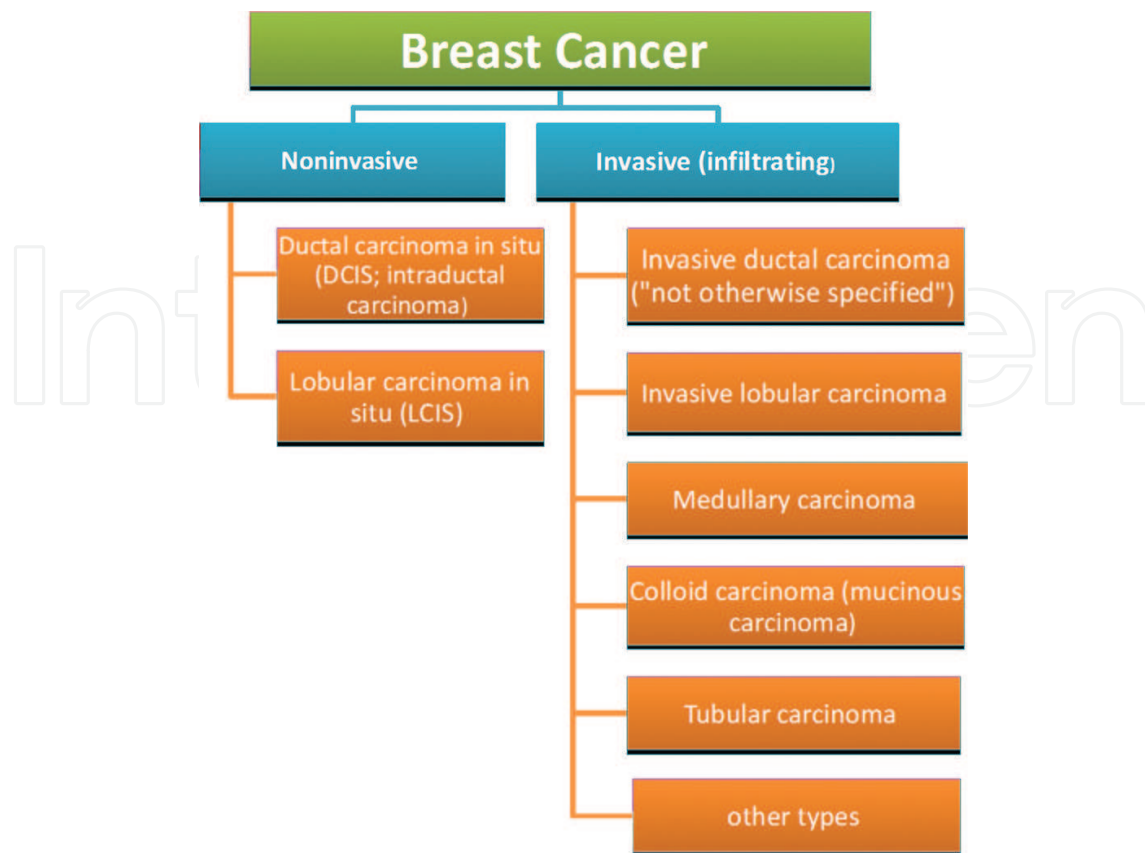


Figure 1. Classification of cancer breast.

2. Breast cancer risk factors

The transformation of the normal epithelium into carcinoma is a multistep process. Genetic background and environmental and dietary factors have a role in breast cancer development. In the normal breast tissue, there is a balance between negative and positive growth factors, so to develop, breast cancer requires loss or gain in some functions [5]. The following factors are thought to be related to breast cancer development:

2.1. Age

The increased incidence of breast cancer with age may reflect the accumulation of somatic mutation. Early menarche and late menopause prolong the exposure to ovarian hormones and are associated with a higher incidence of breast cancer. There is some evidence that breast cancer in younger women is more aggressive than in older women, consistent with a more rapidly evolving disease declaring itself sooner clinically [11].

2.2. Genetic factors

Complex acquired genetic alterations are considered to cause breast cancer, and genetic abnormalities in the premalignant and malignant breast epithelium are likely to have a causal role [12].

That most breast cancers are due to acquired mutations is implied by the fact that only 5% of breast cancer patients have a strong family history indicating inheritance of tumour-promoting mutations in the germ line. Inherited early-onset breast cancer is largely attributable to two genes, BRCA1 and BRCA2. Li-Fraumeni syndrome, ataxia telangiectasia, and Cowden's disease are also associated with increased risk of breast cancer [13].

2.3. Hormonal status

Breast cancer risk appears to increase with exposure to mammotropic hormones, mainly oestrogen, progesterone, prolactin, and insulin-like growth factor 1 during adolescence and adult life. This may be explained by an increased epithelial cell population at risk during the preinitiation stage, affecting clonal expansion and modulating growth enhancement in subclinical tumours. Estrogen is a dominant influence on breast growth, but its role depends on oestrogen receptor (ER) expression in the target tissues. Recently, it has been suggested that overexpression of oestrogen receptors in the normal breast epithelium increases breast cancer risk in women [5].

2.4. Previous benign breast disease

Clear evidence exists that certain subtypes of benign breast disease are associated with breast cancer. In benign breast neoplasia, inactivation of tumour suppressor genes may occur and loss of heterozygosity is also reported. Ductal and lobular carcinomas in situ have a partly malignant morphological phenotype, lacking the ability to invade and metastasize, but are associated with elevated invasive cancer risk. Other lesions associated with abnormal cell proliferation are also associated with more modestly elevated cancer risk, notably the atypical hyperplasia (ductal and lobular) and florid hyperplasia of usual type (that is, without atypia). Frequent coexistence of premalignant lesions with invasive breast cancer is consistent with progression from these lesions to cancer, but there are many controversies in this area, and clonal relationships are not always clear [4].

3. Carcinogenesis and pathogenesis of breast cancer

The prognosis of breast cancer is variable and affected by the heterogeneity of breast cancer, different pattern of breast subtypes, and aggressive genetic behaviour. All these factors may be associated with worsen patient outcome if accumulated with effect of hormonal status, and bilateral oophorectomy may improve prognosis in breast cancer. Depending upon these data, reduced breast cancer mortality is achieved, but still breast cancer is the most prevalent cancer in young women [14]. Aggressive behaviour of breast cancer is due to collision of biologically active tumour and genetic abnormalities, but targeted interventions may improve the survival rate and patient outcome [3].

Estrogen has a crucial role in many tumours including ovaries, endometrium, and mammary gland cancers and also prostate cancer. Estrogen is linked to enhanced proliferation,

decreased apoptosis, and DNA damage in breast cancer. Several experiments on animals have demonstrated that estradiol administration increased the risk of breast cancer, while antioestrogen agents had an opposite effect. Response of breast cancer to antioestrogen therapy after the confirmed presence of high percentage of hormonal receptors is defined as hormonal-dependent breast cancer [15].

The presented framework of circulating tumour cell (CTC) biology and classification of CTC assays might help to structure this dynamic field of translational cancer research. Better insights into the biology of CTCs will further improve CTC assay development [1, 16]. Based on the theories proposed that tumour cells are heterogeneous and breast cancer is the most famous heterogeneous tumour. Therefore, CTC belonging to breast cancer requires special detection approach. Specific profile for CTCs could be targeted for successful detection of complex aggressive breast cancer [17].

Tumorigenesis are postulated by many research works due to multiple steps and may start as chronic disease and processed to cancer. Intervention and prevention of these steps before cancer emerge is a good chance for reducing breast cancer risk. Adjuvant therapies as tamoxifen are effective and safe in significantly reducing and preventing molecular changes that lead to cancer. Those targeted hormonal therapies are very important to stop invasion and metastasis of tumour cells. Blocking DNA mutation is also initiated by using micronutrients and gene therapy to target abnormal pathways that claimed to had a role in carcinogenesis [5].

4. Gene expression profiling of breast cancer

Management of breast cancer depends on clinicopathologic parameters including age, stage, hormonal status, and Ki67 status. Alteration of molecular genetic character including alterations at DNA, RNA, and the protein functional changes contributes to oncogenesis. However, epigenetic changes and regulatory or transcriptional molecules as snRNA, siRNA, and miRNA may be other significantly contributing molecules as well. Successful therapy depends on hormone receptor and human epidermal growth factor receptor 2 (Her2) pathway by analysing their immunohistochemical expression. Failure to respond to the traditional treatment increases morbidity and mortality, and so further discovery of molecular variation in individuals could help in classifying breast cancer subtypes. Clonal analysis of different breast cancer types to understand molecular modifications and genetic expression help in producing full accurate histopathological diagnosis [18].

In the past decade, where breast cancer is clustered in families due to a common genetic factor BRCA1 or BRCA2 mutation, environmental factors shared between relatives may also be relevant. There has been progress in development of new therapeutic approaches that target these BRCA1 and BRCA2 cancer susceptibility genes, which led to loss of functional mutations in either BRCA1 or BRCA2 [19].

Recent delivery of nucleic acid mimics and therapeutic-based miRNA could be used for nano-delivery of target therapy to specific site. In addition, miRNA were recently studied as biological biomarkers in breast cancer, specifically for diagnosis, predicting cancer behavior and outcome [20].

5. Hormone receptors

The breast epithelium undergoes hyperplasia or involution in response to hormone supplementation or withdrawal, and oestrogen and progesterone receptors in the breast tissue mediate proliferative effects. Certain genetic alterations (alteration to the DNA-binding domain) are associated with high overall tumour grade and lack of steroid hormone receptors; an inverse correlation between both receptor expression and nuclear anaplasia also indicates a relation with cellular differentiation. Steroid hormone receptor expression in breast neoplasia has prognostic value. Moreover, there is an independent correlation between oestrogen and progesterone receptor status in breast cancer and tumour progression [21, 22].

5.1. Oestrogen receptors (ER) and progesterone receptors (PR)

Estrogen and progesterone receptors are unique signature to define personalized therapy of breast cancer, and their genetic expression may contribute to breast cancer management through using antioestrogen-targeted therapy. If mutated DNA is spilled from dying cancerous cells into the blood stream, it will become habitant into lymphatic or blood channels and so-called circulating tumor cells. Circulating tumour cells are capable of stimulating other tissues towards continuous proliferation and could be a tool to measure tumour power and ability to enhance metastases [18]. Further research is mandatory to identify those patients at high risk of breast cancer and to understand method of optimization of circulating tumour cell measurement. In addition, there is progressive need for clinical evolution of new agents and targeted therapy, especially to whom with BRCA positive and triple-negative breast cancer patients [23].

5.2. HER2

The HER2/neu oncogene is located on chromosome 17 at band q 21. It is related to the *cerbB-1* (EGFR, HER1) gene which encodes the epidermal growth factor receptor. In addition to its function as a growth factor receptor, it is involved in the regulation of cellular differentiation, adhesion, and motility. When HER2/neu gene is amplified and as a result HER2/neu protein is substantially overexpressed, it is very likely that this plays a role in tumour development and progression [3]. HER2 amplification and overexpression may provide prognostic and therapeutic information in breast cancer and predict resistance to adjuvant therapy. Amplification of this gene is associated with rapid proliferation, shorter disease-free survival, and poorer prognosis in both node-negative and node-positive ductal breast carcinomas and a risk factor for the development of distant metastases. It has true independent prognostic significance but is associated with hormone-independent tumours. Progress of personalized medicine does well for patients to depend on Herceptin treatment. The ultimate goal is to understand cancer behaviour and improve patient survival rate and treatment outcome [6]. Some of the currently utilized cancer biomarkers for breast cancer and their clinical significance are illustrated in **Table 1**.

Biomarker	Clinical utility	References
ER	ER positivity indicates better prognosis in breast cancer patients who have better survival than ER-negative breast cancer patients	[55, 56]
	Predict responsiveness to tamoxifen as when highly expressed, it predicts better response to tamoxifen therapy particularly in node-negative patients	[56]
PR	Prognostic marker indicating better survival when positively expressed (PR +ve)	[55]
	High expression of PR predicts beneficial response to tamoxifen chemotherapy	[57]
HER2/neu	Prognostic marker for worse prognosis in patients with HER2/neu-positive tumours as they have more aggressive breast cancers	[58]
	Predictor marker for the response to therapy with trastuzumab	[59]
BRCA1	Prognostic marker for poor prognosis. High expression of BRCA1 indicates worse prognosis	[60]
	If highly expressed, BRCA1 can predict response to chemotherapy in breast cancer patients	[61]
MammaPrint	Prognosticator in a heterogeneous population for stratification of breast cancer patients into good or poor prognosis, it is a 70-gene assay	[38]
Oncotype DX	A 21-gene multiplex prognostic assay used for determination of recurrence score.	[62, 63]
Isoforms Akt kinase	Akt kinase isoforms and activity are predictive markers to suggest the most likely response to trastuzumab therapy in HER2-neu-positive patients of breast cancer	[64]

Table 1. Currently utilized cancer biomarkers for breast cancer and their clinical significance.

6. Molecular targeting therapy and personalized medicine in breast cancer

Breast cancer is a heterogeneous disease that encompasses subtypes characterized by specific molecular biomarkers: oestrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2) positive, and triple-negative (TNBC) which are ER, progesterone receptor (PR), and HER2 negative breast cancers [5]. Perfect diagnosis and detection of molecular abnormalities help in improvement of personalized therapies to block these mutations by targeted therapy. The best example is using HER2 expression by immunohistochemistry or gene amplification to develop accurate therapy with trastuzumab [24]. Trastuzumab is targeted against domain IV of HER2 [25], while pertuzumab (Perjeta) is targeted monoclonal antibody against the ligand domain II of HER2. Lapatinib is dual targeting therapy for both HER2 and epidermal growth factor receptor (EGFR 1), specifically against its intracellular domain; it acts as tyrosine kinase inhibitor (TKI) as well [26]. Patients on combined lapatinib and trastuzumab have better outcome and survival; such therapy could be assessed with HER2 expression [27]. In fact, combining the HER2 targeting therapies as a neoadjuvant or for metastatic late stage, as trastuzumab and lapatinib or pertuzumab, will significantly improve patient's outcome in comparison with single anti-HER2 therapy [28–30]. Using combined and synergistically acting therapy on the same target (HER2) would achieve better response because of the concomitant action on same receptor against two different epitopes, in addition to the likely deaddiction effect of target that may involve stimulation of the immune system [31].

6.1. mechanistic target of rapamycin (mTOR)/PI3K/Akt-pathway inhibitors

Preclinical studies of the effects of AZD2014 in breast cancer are promising steps to confirm anti-proliferative role of mTOR signalling. Targeting mTOR pathway could suppress the development and progression of cancer, specifically gastrointestinal malignancies and breast cancer. The functions of mTOR pathway are mainly targeted for growth signaling, nutrient status, and metabolism with recent undiscovered impact in obesity development [23].

6.2. Therapeutic cancer vaccine

The monoclonal antibody drugs encourage T cells to detect and destroy cancer and increase the ability of the immune system to respond to tumours. Application of therapeutic cancer vaccine stimulates tumour antigen aiming at activation of tumour-specific T cells [32]. Therapeutic cancer vaccines require the selection of appropriate antigens that are not prone to central immunological tolerance induction in the thymus. Each type of cancer has its own particular immune-suppressive mechanisms guided by information of the immune memory. Selection of the best vaccine and its antigen delivery points should take place and be applied in treatment properly [33]. Breast cancer has the advantage of being based on huge antigen pool for an individual tumour, thus using tumor based-vaccines in breast cancer stimulate the activation of polyclonal immune responses. However, tolerance could occur to the immune system for expression of these antibodies. Co-stimulation and sensitization of the immune molecules will deliver all signals needed for activating T cells under the effect of antigen-specific immune response. Cytokines in breast cancer could be an ideal example of the immune costimulatory molecules [34].

7. "Omics" and promising biomarkers in breast cancer

Recently, with the merging of "omic" technologies such as genomics, proteomics, metabolomics, transcriptomics, etc., a great advancement has been achieved in the field of cancer biology with better understanding of carcinogenesis, cancer progression, metastasis, and target therapy [35]. Microarray, mass spectrometry, and sequencing techniques provide evolutionary era for promising cancer biomarkers [36]. Transcriptional profiling has been reported as a valuable tool for classification and determination of prognosis in patients of breast cancer [37, 38]. Apart from diagnosis, prediction of response to therapy, and prediction of breast cancer patients' outcomes, biomarkers may estimate risk assessment of getting cancer [39]. Genetic alterations in breast cancer or methylation of promoters of cancer-specific or associated genes will definitely linked to altered expression of certain proteins and may be used as emerging cancer biomarkers [40].

7.1. Genomic biomarkers: MammaPrint and Oncotype DX

MammaPrint is one of the emerging genomic assays that have been reported as prognostic biomarker, MammaPrint assay analyses 70 genes' expression signatures, and it is used to stratify patients into good or poor risk groups for recurrence [41]. Another example of promising

genomic markers is the 21-gene signature assessing test, Oncotype DX. It is a quantitative real-time qRT-PCR-based assay, and both assays may provide physicians with very effective prognostic information and consequently would help in selecting early-stage hormone responsive breast cancer patients who will have a likelihood of disease recurrence [38]. Signatures for both assays include genes as ER, HER2, PR-regulated transcripts and proliferation-linked genes that mainly have been utilized as a very effective tool for assessing the probability of recurrence as well as for classifying patients accordingly into high-, intermediate- or low-risk groups for recurrence. In addition, Oncotype DX assay may be used for assessing response to tamoxifen therapy [42].

7.2. Proteomics

Proteomic approach has been investigated through mass spectrometry, two-dimensional gel electrophoresis, and other strategies and successfully has identified promising markers for early diagnosis of ovarian cancer [43, 44]. In spite of being invalidated, their results have paved the path for applying the proteomic approach, via mass spectrometry, for identification of other biomarkers in serum in breast cancer [45] and nipple aspirate as well [46]. Moreover, a panel of proteins has also been identified by high-throughput antibody arrays' technique, and their levels were significantly increasing in malignant breast tissue when compared to normal tissue. Such panel included p53, MAP kinase 7, and casein kinase Ie and annexin [47]. In fact, recent proteomics techniques such as nano-techniques are evolutionally emerging and promising to overcome few limitations of the conventional techniques for identification of potential biomarkers for early detection of cancer; thus, these techniques have to be applied on larger scale of cancer patients and, more importantly, with standardized protocols in order to validate the potentially valuable biomarkers [48].

7.3. DNA methylation

DNA methylation is an example of DNA modification that could be detected and linked with the unique identity of that gene; thus, DNA methylation patterns differ between normal and tumour tissues, and hence, targeting candidate genes could be used to identify and detect cancer cells in the blood or body fluid [49]. Identification of DNA methylation mapping and assays has been applied to nipple aspirate as well for detection of cancer cells at early stage of breast cancer [50]. DNA methylation assessment has been investigated as a prognostic marker for breast cancer in serum, and it was reported that methylation of adenomatous polyposis coli (APC) gene and Ras association domain family 1 isoform A was significantly linked and independently associated with poor outcome in breast cancer patients [51].

7.4. Circulating tumour cells (CTCs)

Circulating tumour cells (CTCs) are detached or disseminated cells from solid tumours or their metastasis into circulation. It has been firstly detected in the bone marrow, called disseminated tumour cell (DTC) patients with early-stage breast cancer [52]. Once CTCs dislodged from cancerous tissues into circulation, they retain the proliferating capacity and ability to settle in other tissues. CTCs have the capability to proliferate and eventually forming metastasis.

Hence, CTC could be a predictor marker for invasion and metastases [18]. Recently, CTCs were considered a dynamic prognostic marker whether in early- or late-metastasizing breast cancer cases [53]. Evaluation of CTCs might contribute for efficient therapy monitoring; in addition, expression profiles of CTCs may predict the likely responses to treatment. As well, assessment of the molecular features of them may be a pivotal step for the optimization of therapy [54].

8. Conclusion and prospective

Breast cancer with its heterogeneous nature and complex behaviour in great needs requires potential biomarkers to improve screening, diagnosis, classification, prognosis, and prediction to therapies. Understanding biology of breast tumour cell, host immune defences, and the tumour microenvironment may allow early detection and recurrence in breast cancer patients.

Breast cancer patients, clinician, pharmaceutical companies, and targeted therapy developer in great needs for flexible, simple, and inexpensive tests with sharp accurate comprehensive diagnosis. The identified molecular aberrations could be arrested and held up by the corresponding targeted compounds, which are best exemplified by detection of HER2neu expression in breast cancer by immunohistochemistry and gene amplification tests for accurate treatment with trastuzumab. Molecular fingerprint for breast cancer generated to help medical practitioner and healthcare providers to focus on patient's prognosis and adopted the preferred best therapeutic option. Designation of distinctive incompatible genetic markers enhances shrinkage of toxic side effect from overuse of therapies and exaggerated gains to patients.

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