Biomedicine & Pharmacotherapy 106 (2018) 14–34

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

Clinical and molecular aspects of breast cancer: Targets and therapies

R.L.N. Godone[a,1], G.M. Leitão[a,b,1], N.B. Araújo[a], C.H.M. Castelletti[a,c], J.L. Lima-Filho[d,e], D.B.G. Martins[a,e,*]

[a] Molecular Prospection and Bioinformatics Group, Laboratory Keizo Asami of Immunopathology (LIKA), Federal University of Pernambuco (UFPE), Brazil
[b] Clinical Hospital of Pernambuco – Professor Romero Marques, Federal University of Pernambuco (UFPE), Brazil
[c] Agronomic Institute of Pernambuco (IPA), Recife, Pernambuco, Brazil
[d] Laboratory Keizo Asami of Immunopathology (LIKA), Federal University of Pernambuco (UFPE), Brazil
[e] Department of Biochemistry, Federal University of Pernambuco (UFPE), Brazil

ARTICLE INFO

Keywords:
Biological markers
Molecular targeted therapy
Breast cancer

ABSTRACT

Breast Cancer is a complex disease characterized by the occurrence of multiple molecular alterations. Currently, some molecular markers are in use for breast cancer diagnostic, prognostic, and predictive purposes. Thus, genetic signatures are available for improving the decision-making. The biomarkers are also essential as therapeutic approaches, but many questions remain due to the lack of efficacy on breast cancer treatment, mainly for triple-negative breast cancer subtype. Since the genetic profile of breast cancer can also be related to different ethnic groups and geographic areas, the reference populations of the genetic assays and clinical trials need to include a broader population beyond the European and North American patients. In this review, we analyzed the current and potential molecular markers that could help to improve the strategies for breast cancer therapy.

1. Introduction

Cancer is a multifactorial disease with a striking heterogeneity due to genetic, epigenetic and transcriptional changes involving a myriad of genes and proteins. While these factors are relevant to clinical prognosis and medical treatment, a broad approach is needed to unravel the complexities underlying carcinogenesis mechanisms [1]. The breast cancer is the second most common cancer in the world and, by far, the most frequent cancer among women. It is the most frequent cause of cancer death in women in less developed regions (324,000), and the second in more developed ones (198,000) [2], but more equally distributed compared to other cancers across regions [3].

Nowadays, we are tackling the precision oncology era whose patients can be treated according to their genetic profile [4]. In breast cancer research, the goal in the field of oncogenomics is to respond to relevant clinical issues related to patients whose tumors will remain inactive for a long time, the appropriate targeted therapy according to the adjuvant scenario, and the most effective approach to improve the life quality of these patients [5]. Despite advances in innovative clinical trial designs, intratumoral and intertumoral heterogeneity persist as challenges [6].

An overview in breast cancer genomes demonstrated remarkable genomic complexity and variability. Individual tumors often carry aberrations that deregulate hundreds or even thousands of genes, which can occur at various levels such as chromosomal, gene replication, transcription, and epigenetics [7]. Although genetic variants in breast cancer can be related to either geographic areas or ethnic groups, the reference populations of the leading commercial tests are European and North American. It is undeniable that development of molecular panels of genetic mutations and gene expression is helping the therapeutic decision, but it is not taking into account all the genetic variability of this neoplasm across the globe. This review summarizes the main molecules studied in prognostic and predictive assays, discusses the molecules used for targeted therapy in breast cancer and underlines new potential biomarkers.

2. Molecular markers in breast cancer

The discovery of the first significant breast cancer susceptibility gene *BRCA1* occurred in 1994, highlighting the inheritance of a mutation in *BRCA1* and *BRCA2* as a risk factor. These tumor suppressors genes are involved in critical functions, like DNA damage response (DDR) and DNA repair [8]. The identification of women at high risk of breast and ovarian cancer is not easy since the loss of one copy of

* Corresponding author at: Av. Morais Rego, 1235 Cidade Universitária – CEP: 50690-701, Universidade Federal de Pernambuco, Laboratório de Imunopatologia Keizo Asami (LIKA), Setor de Prospecção Molecular, Brazil.
E-mail address: bruneska@prospecmol.org (D.B.G. Martins).
[1] These authors contributed equally to the study.

https://doi.org/10.1016/j.biopha.2018.06.066
Received 20 January 2018; Received in revised form 1 June 2018; Accepted 13 June 2018

functioning *BRCA1/2* is not clinically evident [9]. Therefore, the genomic analysis became a predictor tool and most centers developed strategies to reduce cancer risk, morbidity, and mortality in women who carry pathogenic *BRCA1* and *BRCA2* mutations. Then, women should undergo regular screening by imaging to detect tumors at an early stage, risk-reducing mastectomy and/or risk-reducing salpingo-oophorectomy, and chemoprevention strategies [10]. By another side, only 5% of breast cancer development is related to the germline mutations in *BRCA1* and *BRCA2* and few other rare variants [11].

Gene expression profiling is improving the identification of genes whose activity within tumors can provide information on how to assess the prognosis of disease and guide therapy. These gene expression profiles are applicable not only as a prognostic tool but also as a predictor of chemo- and hormone-sensitivity, identifying patients with poor or favorable prognosis, and determining the risk and benefits of adjuvant chemotherapy [12,13].

In 2005, The Cancer Genome Atlas (TCGA) was launched as one of the leading projects of the genetics of cancer, using genome analysis technologies to generate new cancer therapies, diagnostic methods, and preventive strategies, accelerating the comprehensive understanding of cancer [14]. After a decade, more than 11,000 human tumors across 33 different cancer types were available. A standardized dataset called the TCGA Pan-Cancer Clinical Data Resource was developed to ensure proper use of this vast clinical dataset associated with genomic features [14,15]. Since 2016, the data from TCGA project resides in the Genomic Data Commons (GDC), as a research program of the National Cancer Institute (NCI) that provides to the cancer research community the unified data repository, enabling data sharing across cancer genomic studies in support of precision medicine. It is an interconnected database from TGCA, TARGET (Therapeutically Applicable Research to Generate Effective Treatments), International Cancer Genome Consortium, NCI clinical trials, and user-submitted data [16].

Next-generation sequencing (NGS) has made the genomic mutation treatment accessible for breast cancer patients (potentially responsive to targeted therapies), especially in the metastatic setting [17,18]. Several studies have shown advances in the characterization of mutational profiles of breast cancer, as well as demonstrated the importance of inter- and intra-tumor heterogeneity [19]. Large-scale studies on mutation profiles in breast cancer revealed genomic alterations and mutational signatures that can contribute to the comprehension of the mutational landscape, resistance to therapy and strategies for developing better treatments [18–21].

The first study to reveal a molecular classification for breast cancer using molecular taxonomy came from the laboratories of Perou and Sørlie [22]. The authors identified five distinct molecular subgroups of breast cancer using microarrays data: Luminal A, Luminal B; the human epidermal growth factor receptor 2 (HER2)-Enriched, Basal-Like, and Normal-like. They expanded the first classification to include Claudin-low subtype, characterized by low expression of cellular adhesion genes [23] (Table 1).

Clinical oncologists extrapolate Perou's molecular classification of breast cancer subtypes for a more accessible evaluation, aiming to overcome the challenges in applying the molecular finding in clinical routine. In 2011, the St. Gallen Consensus Conference adopted molecular markers estrogen receptor (ER), progesterone receptor (PR) and HER2 for treatment decision-making in early breast cancer for all patients; reinforced years later [24,25].

The high expression of Ki-67 by immunohistochemical (IHC) was also indicated as a marker in breast cancer with good response to neoadjuvant chemotherapy. However, the Ki-67 staining lacks analytical validity, so its performance as a biomarker for prognostic purposes remained weak, with no reliable evidence of chemotherapy efficacy [26]. Therefore, Ki-67 scores should be interpreted in the light of local laboratory values. If a laboratory has 20% median Ki-67 score in receptor-positive disease, values of 30% or above could be considered 'clearly high'; those of 10% or less 'clearly low' [27,28]. As consequence, in broad clinical terms, four subtypes call for distinct treatment approaches: HER2-positive (HER2 + ve) tumors regardless of ER status; TNBC; and two types of ER-positive (ER + ve) breast cancer (Luminal A-like and Luminal B-like) mainly differentiated by expression level Ki-67 protein [26,28,29].

Table 1
Biological classification of breast cancer subtypes according to the phenotypic profiles.

Biological Subtypes	Phenotype
Luminal A-like	ER + ve / PR + ve / HER2-ve / clearly low Ki-67
Luminal B-like	ER + ve / PR + ve or PR-ve / HER2-ve or + ve / clearly high Ki-67
HER2-overexpressed[a]	ER-ve / PR-ve / HER2 + ve (+ + +/3+ by IHQ and/or ISH positive)
Triple-negative	ER-ve / PR-ve / HER2-ve
Basal-like[a]	ER-ve / PR-ve / CK5 + ve / CK6 + ve / CK14 + ve / CK17 + ve / EGFR + ve
Claudin-low	Claudin 3, 4 and 7 low / e-cadherin low/ ER-ve / PR-ve / HER2-ve
Normal-like	Without homogeneous identification

Abbreviations: HER2, type 2 receptor of human epidermal growth factor; EGFR, receptor type 1 epidermal growth factor; ER, estrogen receptor; PR, progesterone receptor; CK, cytokeratin; IHQ, Immunohistochemistry; ISH, in situ hybridization.
[a]True molecular basal like breast cancer and HER2-enriched subtype can be defined by genomic assay only.

Regarding the molecular classification of the Luminal subtype, a distinguishable gene expression signature includes: estrogen receptor 1 (*ESR1*); GATA-binding protein 3 (*GATA3*); Forkhead box protein A1 (*FOXA1*); B-cell chronic lymphocytic leukemia (*CLL*)/lymphoma 2 (*BCL-2*); X-box binding protein 1 (*XBP1*); and the myeloblastosis gene (*MYB*). However, these genes show different profile according to the luminal subtype, as also observed for the mutation profile. Luminal A subtype shows high frequency of mutation in the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*) gene; multiple significantly mutated genes, including mitogen-activated protein kinase kinase kinase 1 (*MAP3K1*), *GATA3*, cadherin 1 (*CDH1*), and mitogen-activated protein kinase kinase 4 (*MAP2K4*); and a low frequency of *TP53* mutation. By another side, high rate of *TP53* mutation was associated with Luminal B subtype together with a slightly lower rate of *PIK3CA* mutation [30].

Overexpression of HER2 occurs in 15%–20% of all breast cancers, associated with aggressive tumor behavior, reduced responses to traditional therapies, and decreased survival [31,32]. However, since 1980, the development of the anti-HER2 class of drugs has improved the outcomes. These agents have notably improved the 5-year survival rate and the overall survival (OS). Therefore, the American Society of Clinical Oncology/College of American Pathologists proposed several recommendations for HER2 test standardization by IHC or *in situ* hybridization (ISH) [24,33].

By another hand, Triple Negative Breast Cancer (TNBC) is more aggressive than other breast tumors and reduces the survival rate of these patients, most often premenopausal women under 50 years [13]. In Mexican patients, ten genes were assigned as associated to TNBC. Nine genes were overexpressed, as follow: protein kinase X-linked (*PRKX*) and protein kinase Y-linked (*PRKY*); UDP-glycosyltransferase 8 (*UGT8*); high mobility group AT-hook 1 (*HMGA1*); lipin 1 (*LPIN1*); hyaluronan and proteoglycan link protein 3 (*HAPLN3*); family with sequence similarity 171 member A1 (*FAM171A1*); B cell CLL/lymphoma 11 A (*BCL11 A*); forkhead box C1 (*FOXC1*); and ankyrin repeat domain 11 (*ANKRD11*). Only one gene, Annexin 9 (*ANX9*), was underexpressed in TNBC samples [34].

However, a molecular stratification of TNBC intends to make easier the development of targeted therapy and the improvement of the patients' quality of life. Applying whole-genome gene-expression profiling