

Breast cancer research: *in vitro* models, markers, and *in silico* analysis - a narrative review

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Abstract

Breast cancer (BC) is one of the most common cancers among women worldwide but can also affect men. Although the detection and diagnosis of BC is advanced, there is a demand for more efficient approaches to treatment. This review summarizes the most important and latest discoveries in the diagnosis and prevention of breast cancer, using scientific discoveries done in vitro cell models, molecular and genetic markers, and data from different in silico analysis studies. Using Google Scholar and PubMed, scientific articles were searched systematically from inception to November 2023. As search terms in this review, we used: breast cancer, in vitro cell lines in BC, genetic and biochemical markers, and miRNA in BC. Based on the literature search, biochemical and genetic markers play essential roles in breast cancer research, whereas in vitro and silico research utilizes breast cancer cell models. Cell models are indispensable tools for the identification of potential new drugs, offering valuable findings on breast cancer growth dynamics. Molecular markers and innovative research methods not only aid in early detection but also contribute to our understanding of BC at various stages, offering treatment strategies and improving outcomes.

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1. Introduction

One of the major causes of death in the world among women is breast cancer and the number of diagnosed patients is increasing every year. Although, the development in detection and diagnosis of breast cancer is evident, there is an essential need for more effective strategies of treatment [1]. Many risk factors are associated with breast cancer development. However, „Western lifestyle“ is mainly and closely related with this type of cancer. Western lifestyle is actually set of habits and practices in everyday life of an individual including excessive nicotine intake, minimal or no physical activity, poor diet, stress and others that can lead to increased incidence of cancer [2].

In the last few years, in vitro research has proven to be extremely important when it comes to the determination of potential cancer treatments. Cell lines which are in vitro models are used in numerous fields from drug discovery to the medical research. Considering the authentic characteristics of specifically selected cancer cell

lines, scientists are able to provide important information such as the effect of potential drugs on the growth and development of cancer category under defined conditions [3-4]. As Breast cancer (BC) poses a significant health concerns, an early detection and effective treatment is crucial for a better outcome prediction. Over the years, various tools and methods have been developed to detect BC in its early stages, leading to improved diagnoses. Understanding the biochemical and genetic markers associated with BC plays a vital role in disease prediction, staging, and treatment decisions. In addition to biochemical and genetic markers, microRNA (miRNA) expression profiling has emerged as a promising avenue in BC research. miRNAs, small non-coding genes approximately 22 nucleotides in length, play pivotal roles in posttranscriptional gene expression regulation. Dysregulation of miRNA expression has been observed in BC patients, distinguishing between normal and cancerous tissues [5]. Finally, the utilization of *in silico* tools in BC research has reached a remarkable level of sophistication. These computational approaches offer significant advantages, including time and cost savings. *In silico* tools enable the prediction of potential drug candidates and their affinity for specific target sites, along with insights into drug metabolism and potential side effects [6].

In summary, the interplay of biochemical and genetic markers, along with the emerging role of miRNA profiling and *in silico* tools, represents a multifaceted approach to addressing the challenges posed by breast cancer. This review will focus on the key studies involving biochemical and genetic markers in BC, miRNAs and *in silico* approach, mainly correlated to breast cancer, in hope to shed a light on their importance in diagnosing and managing this complex disease. If needed, make subdivision for each section as follows.

2. Breast cancer cell lines

Huge portion of the understanding and knowledge about breast cancer is derived from breast cancer cell models [7]. Numerous studies used breast cancer cell models for different purposes. More than 80 different types of breast cancer cell lines are characterized until now. Liu et al. used two breast cancer cell lines, MDAMB-231 and BT-483, and showed that curcumin successfully inhibited proliferation through CyclinD, NF- κ B downregulation and MMP-1 transcription [8]. Furthermore, in various breast cancer cell lines, the HER2-/HSF+ subtypes are shown to be responsive to selected anti-HER2 therapies [9]. Another study, underlines the importance of endocrine resistance in ER+ breast cancer. This study used several breast cancer cell lines, among them BT483, HCC1428 and MDAMB415 for targeting NLK protein (Nemo-Like Kinase) with certain therapeutics and their results showed that therapeutic modulation of NLK is possible [10]. Doxorubicin was used to check the sensitivity of MCF-7 and T47D cell lines, where doxorubicin and NK immunotherapy in combination supported the BC treatment [11]. Further, a research study evaluated effect of phenolic compound on MCF7 cell line and showed that used compound has anti-cancer potential [12]. Trilla-Fuertens and his team in 2018 used CAMA1, T47D, MCF7, MDA-MB-468, HCC1143 and MDA-MB-231 cell lines in order to check metabolic drug response using metformin and rapamycin [13]. Later, Tanjung and Sayekti in 2019 showed that Ancorina sp. extracts can induce apoptosis on T47D cell line [14]. Hasson et al. managed to induce apoptosis in BT474 cell line using lyophilized camel's milk [15]. HCC1419, BT474, EFM-192A, MDA-MB-361 cell lines were tested with combinatorial treatment of trastuzumab and tamoxifen where three out of four showed increased response [16]. Contactin1 (CNTN1) was proved to be important for metastasis in different tumor types, as a study conducted in Hs578T cell lines showed. According to their results, CNTN1 overexpression induced growth and invasion in used breast cancer cell line [17]. Grant et al. in 2018, used several common breast cancer cell lines and reported that among all studied cells lines, BT-549 and Hs578T cell lines have higher sensibility to englerin A [18]. Further, in a different study, curcumin and letrozole were delivered to the cell via magnetic niosomal carrier (NiCoFe₂O₄ coated with layer of silica), increasing the rate of apoptosis in MDA-MB-231 and SK-BR-3 breast cancer cell lines [19]. JIM1, HCC1954 and 21MT1 cell lines were used to evaluate proliferation levels caused by GRB7 knockdown. GRB7 knockdown affected the breast cancer cell lines either by reduced proliferation or increased apoptosis [20].

3. Biochemical and genetic markers in breast cancer (BC)

The best prognosis for breast cancer (BC) patients is associated with early detection and effective treatment of the disease [21]. In order to detect malignancies in their early stages, detection tools and methods must be rapid, accurate, and reliable [22]. Looking back four decades, we can trace the initial adoption of mammography screening, which significantly accelerated the diagnosis of breast cancer. This advancement was particularly invaluable for women aged over 50 years. As previously said, early diagnosis is very important part of the disease treatment since it has been proven that early detection of BC is highly curable and survival rate for 10 years is higher than 97% [23]. BC types are classified according to the location, and we can divide them into 2 main groups. First group belongs to tumors that start to develop in breast ducts represents 80% of all BC types, and another group that starts to develop inside the lobes and it represents 15% of all BC types. Other subtypes belong to the types of BC that are diagnosed in less than 5% of all cases. BC is complex disease which can be divided in the following stages: ductal hyper-proliferation followed by the development of cancer in situ that results in invasive carcinoma and the last stage of BC is metastasis [24, 25]. With the knowledge related to these stages of BC, we can realize how markers for the disease detection are important. Molecular markers are widely used for disease prediction as well as for determination of the stage of cancer, therapeutic response evaluation, disease recurrence detection and detection of metastasis together with providing prognosis related information [26].

3.1. Biochemical markers

3.1.1. Estrogen and Progesterone receptors

Estrogen (ER) and progesterone (PR) receptors are the ones that are studied the most in the BC. It is known that risk factors for BC are strongly connected with ER positive and PR positive subtypes, since they are closely related with the mechanisms that are associated with ER and PR. On the other side, cause for negative ER and PR subtypes in BC needs to be independently assessed, or not related to the exposure to ER and PR due to the low occurrence in BC [27]. According to the literature, more than 700.000 women worldwide are diagnosed with BC (+) hormone receptors (HR) [28]. By the definition, HR are expressed proteins located in epithelium and in breast stroma. They bind to hormones that are circulating through the body and, in that way, they are influencing their cellular effects. ER and PR are mostly related to the patient age, so according to the literature, younger patients have higher chance to be ER [26, 29].

3.1.2. Human epidermal growth factor receptor 2 (HER₂)

If any of the authors has any financial and non-financial competing interest, they must be declared in this section. HER2 has been studied since 1987 when it is confirmed that the poor prognosis is associated with increasing levels of it in the patient body. It is known as a transmembrane tyrosine kinase receptor, epidermal growth factor receptor family [26]. When HER2 undergoes overexpression in the body, it leads to a weak prognosis as it concurrently initiates resistance to both anti-hormonal and cytotoxic treatments. Furthermore, it is related to very aggressive phenotype of the tumor cells and low survival rate [29-30]. So far, numerous studies have confirmed HER2 involvement and overexpression in BC [31-33].

3.1.3. Ki 67 antigen

Ki 67 antigen is a labile, nonhistone nuclear protein which is related to the cell cycle since it is expressed in all phases including G1, S, G2 and M phases [34]. Ki 67 score is usually measured during histological sections following immunohistochemistry process. After the calculation of the stained cells of the invasive carcinoma, final percentage of them are considered as Ki 67 score [26]. This method is considered as precise, especially for the tumor proliferation index estimation and it could be considered as predicting factor in therapeutic decision-making [29, 35-36].

3.1.4. Carbohydrate 15-3 and Carcinoembryonic antigens (CA15-3 and CEA)

In a case that BC is detected by using CA15-3 and CEA antigens, metastases are no longer treatable. CEA represents a glycoprotein which is expressed in a patients diagnosed with BC, and it can give better picture about size of the tumor and the number of lymph nodes that are involved [26, 29]. However, a study conducted in 2020 showed that in patients with triple-negative breast cancer (TNBC), CEA and CA15-3 elevated preoperative levels were not statistically significant prognostic factors for overall survival [37]. CA 15-3 represents transmembrane protein which is overexpressed in more than 90% of all diagnosed BC cases [38]. Several studies have confirmed that elevated CA15-3 and CEA showed to be statistically correlated to low survival rates in BC patients [39- 41].

3.1.5. Carcinoma antigen 27.29 (CA27.29)

CA27.29 is a carbohydrate-containing protein, also called BC-associated antigen [42]. In addition to breast cancer, elevated levels of the CA 27.29 antigen are also detected in other types of cancer, including kidney, liver, and ovarian cancer [26, 43]. Elevated CA 27.29 levels are present in over 80% of all diagnosed breast cancer cases, and one of the challenges associated with this antigen is its limited ability to accurately determine the cancer stage [20, 42]. In the Iraqi population, a study concluded that CA 27.29 antigen is a strong marker for BC development [43]. Further, similar studies indicated the essence of CA 27.29 antigen in BC survival as it decreases after chemotherapy [44]. In general, it is widely acknowledged today that Carcinoma Antigen 27.29 serves as a robust biochemical indicator in the progression of breast cancer [45-46].

3.2. Genetic markers

Between 5 and 10% of all BC cases are reported to be hereditary [38]. Breast Cancer 1 gene (BRCA1) and Breast Cancer 2 gene (BRCA2) are commonly related tumor suppressor malignant genes, where more than 80% BC cases are inherited [48]. Furthermore, BRCA mutations are reported to be highly related with breast and ovarian cancer hereditary syndrome [49]. Research findings indicate that individuals carrying BRCA mutations have a high risk of BC, ranging from 69% to 72%, and they also have a great risk (10-30 times higher) of developing ovarian cancer in comparison with general population [49]. On the other side, within families where a history of breast cancer is well-documented, especially if it's diagnosed at a younger age, the risk of developing breast cancer due to a BRCA1 mutation can reach as high as 90%. In families with a predisposition for both breast cancer and ovarian cancer, BRCA2 testing is often considered more practical and informative [50]. It's proven that mammography testing doesn't prove highly effective for individuals carrying the BRCA mutation. A research indicates that nearly 30% of new cancer cases went undetected through mammography, as it exhibits limited sensitivity in such cases [50-51]. Although BRCA1 and BRCA2 are the most common tumor markers, there are other genetic markers correlated to BC developments, as presented in Table 1.

Table 1. Genetic markers for BC.

Genetic marker name	Explanation	References
Tumor protein p53 (TP53)	Additional confirmation after BRCA testing	50, 52
E-Cadherin(CDH1)	Detected in Lobular BC, confirmed to be overexpressed	50, 53
Serine/threonine kinase (STK11)	mutations have 50% chance to develop BC until the age of 60	50, 54
Checkpoint kinase 2 (CHEK2)	deletion mutation increases the risk for BC	50, 55
Ataxia-telangiectasia mutated (ATM)	mutation increases the risk for BC	50, 56
Partner and localizer of BRCA2 (PALB2)	mutation increases the risk for BC	50, 57
BRCA1 interacting helicase 1(BRIP1)	mutation increases the risk for BC	50
DNA repair proteins RAD51C and RAD51D	mutations increases the risk for BC	50, 58
BRCA1 Associated Ring Domain 1(BARD1)	mutations increases the risk for BC	27, 59

4. miRNA expression profiling

Micro RNA (miRNA) represents a group of small non-coding genes (~22 nucleotides in length) which are responsible for the regulation of posttranscriptional gene expression. This process is achieved through the specific interaction with 3' UTR of target mRNA. The outcome of this process is translation inhibition and degradation of mRNA [60]. It is noticed that miRNA has essential role in tumorigenesis and that there is a huge difference between expression levels of miRNA in normal and cancerous tissues in patients with BC [61-62]. Human BC cell lines are also great experimental models since they are renewable resources. miRNA is associated with BC pathology characteristics such as ER and PR receptor expression, stage of the tumor, etc. In this regards, miRNA also serves as marker for BC [63-64]. Important to mention is that BC cell lines have preserved major genomic characteristics found in clinical breast cancers [62-65].

Further, a recent study showed that global cell free miRNA levels were significantly correlated with cancer relapse. They reported increase of free miRNA levels in the plasma before the clinical detection of progressive disease and enormously elevated levels in died patients [66]. In a review study, conducted by Gonzales et al. in 2023, proposed a genetic signature including a total of 5 up-regulated miRNAs in metastasis compared with early stages. Two of them, were exclusively present in relapse metastasis, miR-23b and miR-200c [67].

Further, in 2023, a study conducted on Egypt population, revealed that that HOTAIR/miR-1246 exerts an oncogenic impact in patients with breast cancer [68]. After an *in silico* meta-survival analysis, miR-29c and mir-361 have shown the potential to serve as prognostic biomarkers [69].

5. *In silico* approach in breast cancer treatment

Nowadays, the utilization of *in silico* tools has reached an impressive level, as this approach offers significant time, energy, and cost savings. It's truly remarkable how the *in silico* method allows us to forecast potential drug candidates and their compatibility with specific target sites, predicting their metabolism with minimal side effects [6, 70]. Numerous anticancer potential *in silico* studies have so far been published, indicating a huge potential *in silico* approach. For example, as shown in a study combining *in vitro* and *in vivo* approaches, MeOH extract of the shoots of *M. sinaica* negatively affected the growth of MDA-MB-231 triple negative BC cells leading to induction of apoptosis [6]. Furthermore, a molecular dynamic (MD) simulation study validated the stability more than 40 complexes from *Foeniculum vulgare* Mill, where only α -pinene showed enormous potential for the BC treatment, however *in vitro* and *in vivo* studies are required in order to validate the data presented [71]. Thanks to *in silico* approach, authors were able to complete docking analysis, determine protein targets, and define binding energy and to visualize their results. Today, we have more and more studies that are using phytochemical as a potential drug for the BC treatment. An example, an *in silico* molecular docking and physicochemical property study on effective phytochemicals, filtered the G protein-coupled receptor 116 (GPR116) as an effective drug target in breast cancer treatment [73]. Another example, a study analyzed *Epigallocatechin gallate* (EGCG) from green tea, concluding that the NOTCH (1–4) and P53 proteins showed promising results as repurposing drugs for BC treatment [73].

6. Conclusion

In conclusion, breast cancer is a major global health concern, and the number of cases diagnosed each year is on the rise. Although a huge progress in detecting and diagnosing breast cancer is made, there's a pressing need for better ways to treat it. In recent years, lab research using several breast cancer cell models is essential in finding potential treatments. Breast cancer cell line models have unique qualities that help us understand how possible drugs affect cancer growth in controlled settings. It is known that early diagnosis and treatment efficiency is crucial for the improvement of breast cancer outcomes. Certain markers in the body, like hormone receptors (estrogen and progesterone receptors), HER2, Ki-67 antigen, and other substances like CA15-3, CEA, and CA27.29, provide important information for predicting the disease, determining its stage, and choosing treatments. These markers also help us predict how well treatments will work and if cancer might come back.

Other molecular marker, genetic markers, like BRCA1 and BRCA2 mutations, are linked to breast and ovarian cancers that run in families. Additionally, studying small genetic molecules like miRNAs, has become a promising way to understand breast cancer, differentiating normal from cancerous tissue. In the world of computer modeling, advanced tools and methods are helping us make significant progress in breast cancer research. These computer-based approaches save time and money and allow us to predict which drugs might work, how they'll interact with specific targets, and what side effects they might have. To sum it up, a combination of markers in the body, miRNA studies, and computer tools is helping us tackle the challenges of breast cancer from different angles. This review emphasizes how these pieces of the puzzle are crucial for diagnosing and treating this complex disease and highlights our ongoing efforts to find better ways to fight breast cancer.

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