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GENETICS AND MOLECULAR MUTATIONS IN BREAST CANCER

Saliha Khalid[1*], Tabinda Razzaq[2*], Afriaz Khan[3], Sadia Afzal[4], Shiv Ram Ashraf [5], Laraib Zafar[6], Summayya Maryam[7], Kashif Nawaz[8], Abbas Shahid[9]

[1*]Department of Material Science and Nanotechnology, School of Engineering and Natural Sciences, Kadir Has University, Cibali Campus Fatih 34083, Istanbul Turkey.
[2*]Faculty of Pharmaceutical Sciences, Lahore University of Biological and Applied Sciences, Pakistan
[3]Institute of Zoological Science, University of Peshawar, Pakistan.
[4]Department of Zoology, Government College Women University, Faisalabad.
[5]Department of Biochemistry & Biotechnology, University of Gujrat Pakistan.
[6, 7, 9]Department of Biochemistry, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan.
[8]Department of Zoology, University of Okara.

***Corresponding Author(s):** Saliha Khalid & Tabinda Razzaq
Email (s): salihamugha80@gmail.com, & tabindarazzaq@gmail.com

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ABSTRACT
Breast cancer (BC) is still the most commonly occurring malignancy in women and it poses a formidable public health challenge worldwide. It comprises a group of molecularly heterogeneous diseases in patients with a family history and/or suggestive personnel, and a predisposing gene is identified in <30% of patients in this type of malignancy. About 25% of heritable cases are due to a mutation in one of the few identified rare, but highly penetrant genes (BRCA1, BRCA2, PTEN, TP53, CDH1, and STK11), which confer up to 80% lifetime risk of BC. Additionally, 2%–3% of BC cases are due to a mutation in moderate-penetrance gene (e.g. CHEK2, BRIP1, ATM, and PALB2), and each gene is associated with a twofold increase in risk. BCs can begin in different areas of the breast, such as the ducts, the lobules, or the tissue in between. Within the large group of diverse breast carcinomas, there are various denoted types of BC based on their invasiveness relative to the primary tumor sites. For treatment of BCs, personalized cancer vaccination strategy can be an effective approach to trigger a broad-based antitumor response that is both beneficial and relevant to individual cancer patients. However, mRNA provides a template for the synthesis of any given protein, its fragment and lends itself to a broad range of pharmaceutical uses, including cancer immunotherapy. With the ease of rapid, large scale manufacturing and production, mRNA is ideally poised not only for off-the shelf cancer vaccines but also for personalized neoantigen vaccination. This review provides a comprehensive survey of the genetics, molecular mutations, and state-of-the-art information on vaccine-based therapeutics for BC.

Keywords: Breast cancer, BRCA, Family history, genetics, Oncogenes**,** Vaccines, mRNA therapeutics.

Introduction

It is recognized by professionals that malignancy is the consequence of irregular and/or inheritable genetic mutations in germinal or somatic cells. Similarly, breast cancer (BC) is due to both genetic and environmental factors resulting in accumulation of mutations in some important genes [1, 2]. Generally, BC risk increases with age, however, certain non-genetic factors can cause wide variation on the age of onset of the disease [1, 3]. BC is the most prevalent cancer in females and accounts for 30% of female cancers [2, 4]. BC is a heterogeneous syndrome on the molecular level with mortality-to-incidence ratio of 15% [2]. About 10% of women that suffer BC have a familial record of the illness [2]. When women with a family history of BC were compared with those without a family history, those having one first-degree relative with BC before menopause were found to be at 3.3-fold greater risk, and those with two first-degree relatives with BC were found to be at 3.6-fold greater risk, indicating the influence of germline genetics on the risk of disease progress [3, 4, 5].

Even though studies have confirmed about 20 % of the familial risk of BC, they couldn't comprehensively elucidate the role genetics play in non-familial BC [2]. Further studies based on genome-wide association studies (GWAS) have found over 80 loci with significant association to sporadic BC, nevertheless, these variants were only able to give insight into 16 % of BC heritability [4]. Different treatment ideas have been coined to take this heterogeneity into account in last 10 to 15 years, with emphasis upon use of biological-directed therapies to lessen the harmful outcomes of cancer treatment [4, 6, 7]. Regardless of the intrinsic molecular heterogeneity, that is a fundamental standard of existing therapies, few characteristics such as the metastatic patterns or influence of locoregional tumour burden are common and they influence treatments. Most early BCs are asymptomatic and they are confined in the breast or only metastasize to the axillary lymph nodes and are revealed during screening mammography — are considered curable [3, 4, 8, 9]. Advancements in multimodal treatment strategies have dramatically improved survival (~70–80%) and reduced local recurrence [5].

On the other hand, metastatic cancers are considered incurable using currently available therapeutic options. Conversely, metastatic BC is a curable syndrome, for which the foremost objectives of treatment are to increase the survival rates and control deteriorating symptoms of the patients with low treatment-associated toxicity to improve quality of life.

Advancements in cancer research and treatment, the innovative application of heterogeneous photocatalysis has shown potential in environmental and medical fields. Recent studies, such as those focusing on the enhanced decomposition of pharmaceuticals using TiO_2 and ZnO, illustrate the broader implications of catalytic processes which might offer insights into novel therapeutic strategies or diagnostic tools for cancer management [83]."

"The green synthesis of silver nanoparticles using Agaricus avensis has not only shown improvements in catalytic efficiency for enzymes like tyrosine hydroxylase but also holds promise for applications in cancer research. Enhanced catalytic activity could potentially lead to better detection methods or novel therapeutic approaches for breast cancer by targeting specific mutations or pathways involved in tumor progression [84].

As BC is a worldwide health issue, main emphasis needs to be put on reducing worldwide inequalities in access to diagnosis, multimodal treatment and novel drugs. In the present review, authors provide state-of-the-art information on the epidemiology, mortality, risk factors, classification, genetics, molecular mutations, and treatment strategies of early and metastatic BC, highlighting the necessity for multidisciplinary management of this heterogeneous disease.

Demographics, incidence, mortality, and risk factors

Breast carcinoma accounts for 30% of women carcinomas with 15% mortality-to-incidence ratio and is leading oncological cause of death in females around the world [2, 10]. In 2020, there were estimated 19.3 million new cases of cancer and 10 million deaths in the world and BC was highest

prevalent cancer among all cancer sites [2]. Similarly, in 2020, there were estimated 2.2 million cases (2.1 million in 2018) of BC with 684,996 deaths (629,679 in 2018) worldwide [2]. The worldwide incidence of BC has been rising annually, beginning with 641,000 cases in 1980, >1.6 million in 2010 and 2.1 million in 2018; this rising trend is likely to continue [2]. Among 36 different types of tumors, female BC is the most commonly detected tumor worldwide. 49.6% of global population accounts for females, and females aged >60 make a larger proportion of the population [2]. The death rates of subtypes of BC also vary with HER2-positive disease associated with a higher death rate, followed by the TNBC, luminal A and luminal B subtypes [2].

BC worldwide incidence varies between high-income regions (97 in 100 000 in North America) and low- and middle-income regions (LMIRs) (27 in 100 000 in east Asia and Africa) [6, 11]. However, mortality is usually higher in LMIRs because of late stage diagnosis, delayed presentation, and limited access to advanced treatments [6, 11, 12]. Moreover, BC presents earlier in Asian females (at the age of 40-50 years) as compared to western females (at the age 60-70 years) [6]. Numerous studies have looked at contributing factors such as socioeconomic level, health-care access, and genetics [7, 13]. Poor outcomes are partly caused by socioeconomic and health-care access disparities but tumour biology that is more aggressive also plays a key role [6, 7, 14]. Tumour biology also differs in ethnicity and race, and this aspect has implications for the difference in mortality. For instance, African-American females had the highest incidence of TNBC than any other ethnic group. Black women are more likely to be diagnosed with advanced-stage cancer, and TNBC harm them disproportionately [15, 16].
There are many factors which are attributed to higher number of BC cases which include pregnancy-associated factors, hormonal therapy, lifestyle factors (i.e., smoking, physical inactivity, low-fibre diet, obesity, and alcohol intake) and some additional risk factors (high breast density, low number of births, late pregnancy, short or no breastfeeding, older age, and birth control contraceptives) [17]. Hence, more than third of cases could be preventable through lifestyle changes. Moreover, menopausal hormone therapy, has been shown to enhance the risk of BC [8, 18, 19].

Classification of BC

There are different types of BC as it can occur in distinct areas of the female's breast (ducts, lobules, or the tissue in between) [1]. BCs can be divided into two general classifications, sarcomas and carcinomas on the basis of cell origin involved [1, 4, 20]. Sarcomas are rare form of BC (<1%) which arise from the stromal components (myofibroblasts and blood vessel cells) of the breast [1, 3]. On the other hand, carcinomas are common type of BCs arising from the epithelial component of the breast. Epithelial component consists of the terminal ducts (used for making milk) and cells that line the lobules. Most types of BC are carcinomas [1]. In carcinomas group, there are various types of BCs which are recognized on the basis of their invasiveness relative to the primary tumor sites **(Figure 1)**. On the basis of pathological and invasiveness characteristics, breast carcinomas can be categorized into three groups: invasive, non-invasive, and metastatic BCs [1, 3, 4, 21, 22].

Non-invasive malignancy

Intraductal malignancy is very common type of BC which develops within the pre-existing healthy and normal ducts. As this type of malignancy is not invasive but cancerous cells have the potential to become invasive. Hence, timely diagnosis and treatment are very crucial to prevent invasive cancer [1].

Invasive malignancy

Invasive BCs are composed of cancerous cells that invade and spread outside of the normal breast lobules and ducts, growing into the surrounding breast stromal tissue [1]. This type of cancer is diagnosed in women after the age of 55 years or older. Invasive malignancy has the potential to metastasize other sites in the body (e.g., lymph nodes and other organs) and thus can be classified as metastatic BCs. On the basis of cell types and tissue involved, invasive BC can be further divided into

two types: Invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC) [1]. IDC is the most common type of BCs and about 80% of all BCs constitute invasive cancer carcinomas. Several subtypes of IDC are present including medullary carcinoma of the breast, papillary, tubular, mucinous, and cribriform carcinoma of the breast. After IDC, ILC is the second most common type of BCs and accounts for approximately 10-15% of all BCs [1]. It is more common in older women. Both types of invasive breast cancer indicate distinct pathologic characteristics and almost 90-95% patients fall into invasive category [1, 4, 9, 23, 24].

Metastatic breast cancer

These are advanced or stage VI BCs, which have metastasized to other/distant sites of the body [9, 10, 25]. Metastatic cancerous cells can be present in distant sites including brain, bone, lung and liver or may be detected in armpit (in lymph nodes). It is possible for the metastatic cancerous cells to remain in the body even after the removal of the primary tumor, allowing the cancer to reoccur and propagate **(Figure 1)**. However, the mechanism and risk of recurrence and metastasizing is not inferred very well because it differs from person to person, and depend on the stage at time of diagnosis with distinct molecular biology of the tumor [11, 26, 27].

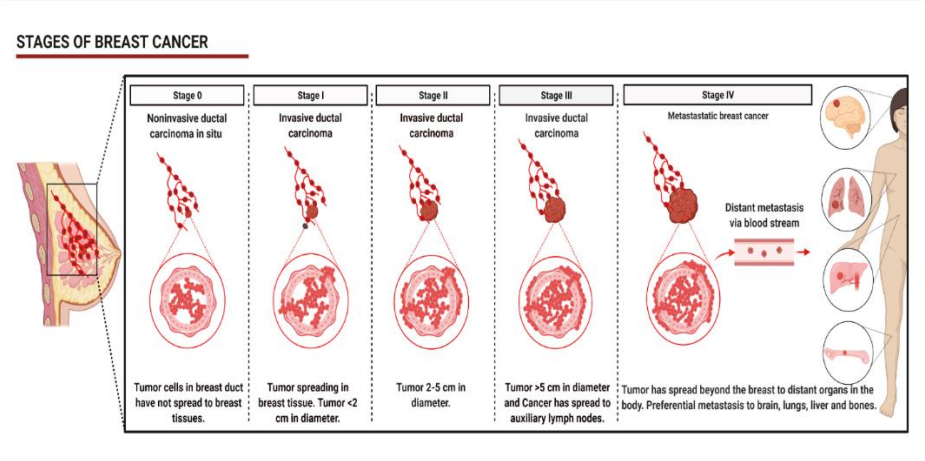

Figure 1. Stages of breast cancer.

At stage IV BC, malignant tumors typically give rise to metastases, making the cancer hard to eradicate. The drawing shows different stages of BC and common sites in the body for metastases from carcinoma of the breast**.**

Genetic Penetrance and Breast Cancer

Several studies have reported that mutation in some types of genes puts patients at more risk of BC than mutation in other types of genes. Most of the familial cases of BC are as a result of rare but high penetrant genes but other rarer genes classified as moderate- and low-penetrance alleles have also been found to play an important role in BC initiation [12, 13, 28].

High Penetrant Genes

In people with family and/or personal history of BC, about <30% of patients are identified with particular predisposing gene while about 25% of heritable cases were caused by a mutation in one of the few rare genes that are categorized as highly penetrant [13].

These highly penetrant genes include PTEN, STK11, TP53, BRCA1, BRCA2 (BRCA1/2), and CDH1, which could be responsible for about 80% lifetime risk of BC. Furthermore, approximately, 2%–3% of BC cases are because of mutation in rare genes classified as moderate-penetrant genes. These genes include PALB2, CHEK2, ATM, and BRIP1, each of these genes are linked with a two-time increase in risk of BC [2, 13].

The mutations in BRCA1/2 are inherited in an autosomal dominant fashion, but act in a recessive manner at the cellular level as tumor suppressor genes involved in double-stranded DNA (dsDNA) break repair [4, 13]. It has been estimated that carriers of mutations in BRCA1/2 among female are susceptible to a lifetime risk of BC of 50% to 85%, however, males carriers of the BRCA1 have an increased risk although lesser than carriers of BRCA2 who have up to 5%–10% lifetime risk. Mutations that include deletions and rearrangements in BRCA1/2 are found to account for only 15% of hereditary BCs [2, 3 5, 13, 20, 29].

Moderate-penetrance Genes (MPG)

The introduction of genome-wide association studies (GWAS) has opened doors for novel discoveries wherein more than 80 moderate-penetrance and low-penetrance variants were discovered. While the scientists to study these mutations were very lucky for discovering variants associated with sporadic as well as hereditary BC, the variants identified could only elucidate 50 % of the heritability of BC [13]. On the basis of identified cellular functions in families with possible exposure to BC, many studies have been carried out on specific genes said to increase the risk of BC. Such identified genes have extra DNA repair genes such as PALB2, CHEK2, ATM, and BRIP1 (BACH1), and they interact with BRCA pathways we well as BRCA1/2, and cause nearly twofold higher risk of BC [13, 30].

BRIP1

It is also known as BACH1 and FANCJ is a gene that encodes a protein that interacts with the BRCA1 C-Terminus (BRCT) domain of BRCA1 [1, 13]. Observed mutations in BRIP1 are said to cause that amount to less than 1% cases of BC. The prevalent type of the mutation observed in BRIP1 is protein-truncating mutations. Mutations in in this gene also confer high risk for ovarian cancer in women.

ATM

It is a serine-threonine protein kinase whose function is to repair and monitor dsDNA and to regulate CHEK2 and BRCA1. The prevalence of monoallelic ATM mutation was estimated to be around 1%. It has been reported in a meta-analysis that the relative risk of BC linked with ATM mutation was high (i.e., 2.3), with a greater risk observed among females of less than 50 years of age. Mutations in the MPGs may be influenced by some factors (e.g., environmental) reducing their genetic impact. It has been reported that different selenium concentrations have been shown to affect the ATM-and CHEK2-dependent DNA repair mechanisms/pathways [2, 3 5, 13, 20, 29].

Partner and localizer of BRCA2 (PALB2)

It is a gene that encodes a protein that can performs its functions by associating with BRCA2 and this association is compulsory for DNA damage repair. The main function of PALB2 is stability of the cell and nuclear localization. Estimates showed 1%–2% incidence and RR of 2.3 for all women and RR of 3.0 in females who are under 50 years of age. Generally, the moderately penetrant genes cause lower lifetime risk of BC than the highly penetrant genes [2, 3 5, 13, 20, 29].

Low-penetrance Alleles (LPA)

Reduced penetrance of alleles is a widespread phenomenon in genetics and it has been detected with most sophisticated genetic testing techniques. Although they may add to BC risk in a polygenic manner, their identification could still be vital in a certain cases even if they may not be used in routine practice.

Mutations in Genes Responsible for Breast Cancer

Most of the BC cases are due to primary mutation in oncogenes which results in uncontrolled cell propagation. Moreover, mutations in tumor suppressor genes (TSGs) have also been implicated in the cause of malignancy. Hereditary BC which account for 5%–10% of all cancer cases is as a result genetic mutations in certain genes susceptible to cancer. The current review examines the mutations that takes place in some genes, such as tumor suppressor genes, oncogenes and other cancer

susceptible genes that are accountable for cell growth and proliferation [1, 13, 30, 31]. Molecular subtypes of BC are given in **Table 1.**

AR: androgen receptor; CDH1: E-cadherin; CLDN: claudin; CK: cytokeratin; EGFR: epidermal growth factor receptor; HER: human epidermal growth factor receptor 2; ER: oestrogen receptor; PR: progesterone receptor; - : negative; +: positive; +/-: occasionally positive; -/+: rarely positive.846

Table 1. Biological Characteristics of breast cancer molecular subtypes and assignment of histological special types of breast cancer.

Biological BC subtypes	Common histologic types	Molecular signatures	Outcome	Additional markers	Ki67 (by IHC)	Histological grade
Luminal A-like	Lobular, classical, tubular, cribriform	ER +, PR +, HER2-, clearly low Ki-67	Good	-	Low	1|2
Luminal B-like	Micropapillary	ER +, PR ±, HER2±, clearly high Ki-67	Intermediate/poor	-	High	2|3
HER2-overexpressed	plemorphic	ER-, PR-, HER+	Poor	CK5/6+/- ,EGFR+/-	High	2|3
Triple-negative	Metaplastic, adenoid cystic, secretory	ER-, PR-, HER2-	Poor	AR+, CK5/6+/- , EGFR+/-	High	-
Claudin-low	Metaplastic, medullary	Claudin 3, 4 and 7 low, e-cadherin low, ER-, PR-HER2-	Poor	CLDN low-, DH1 low-, CK5/6+/- , EGFR+/-	High	3
Basal-like	Medullary, metaplastic, adenoid cystic, secretory	ER-, PR-, CK5+, CK6+, CK14+, CK17+, EGFR+	Poor	CK5/6+ , GFR+	High	3
Normal-like	Medullary, Metaplastic	Without homogeneous identification; ER+, PR±, HER2-, Low Ki67	Intermediate	CK5/6+ , EGFR+	High	1|2|3

Oncogenes

Oncogenes are mutated and primary genes that have been extensively investigated at the molecular level and they contribute to the development of cancer [13, 14]. Oncogenes are said to represent changes in the proto-oncogenes that are unmutated and play their roles in the normal regulation of cell division, proliferation, and growth.

Genetic mutations in these genes causes gain-in-function or cell growth and proliferation because they are responsible for activating the cell for active cell division [14, 15, 32].

These genes are accountable for the majority of BCs because a single alteration can activate them, amplify or even overexpress their protein products. Even though oncogenes are responsible for cancer initiation, their influence in the later stages of the cancer is insignificant.

There are a number of oncogenes involved in the initiation of BC out of which c-MYC, HER2, and RAS are among the most studied oncogenes. Apart from the oncogenes, other genes that are responsible for malignancy include cyclin D1 and E, estrogen receptors (ERs), and cyclin-dependent kinases 2 [13, 14, 33, 34].

Human Epithelial Receptor 2 (HER2)

HER2 (also called as c-neu or c-erbB2) is a protein that helps BC to proliferate rapidly [17]. It belongs to the human epithelial receptor gene family, among them, epidermal growth factor receptor (EGFR) was the first to be discovered [16, 17, 35].

HER2 produces heterodimers with HER1 and HER3, which is implicated for cancer initiation and formation (**Figure 2**). The stimulated dimers are subsequently involved in various molecular pathways like P13K/AKT/mTOR and MAPK pathways through signal transduction (**Figure 2**).

The main roles played by the HER receptors are primarily angiogenesis, metastasis and resistance to apoptosis, altered cell-cell interactions, cell growth and propagation, and increased cell motility [14, 15]. HER2 is involved in the activation of almost half a dozen genetic pathways responsible for proliferation and growth of the cell.

The HER2 gene in BC cases is said to be amplified by 20%–30% or the HER2 protein is overexpressed in around the same percentage except in some cases where the protein was found to be overexpressed without the gene amplification [13, 14, 15 ,17, 36-39].

Overexpression of HER2 is mainly found in BCs of ductal origin and not in the lobular origin. HER2 has been reported with higher recurrence rates and it exhibits poor response to different therapies like chemotherapy and hormone therapy as well as overall reduced diagnosis and survival.

It was also reported that 15% of BC patients expressed poor levels of this protein compared to the healthy tissues of breast and also displayed high-grade cancer than patients in which the protein is overexpressed [40, 41].

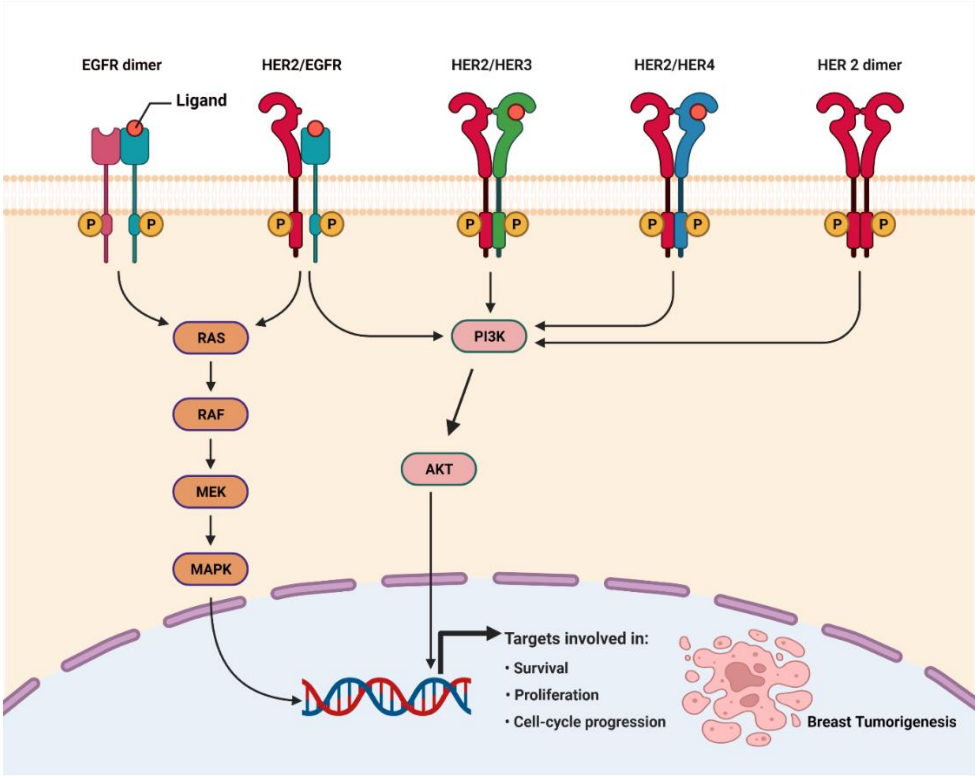

Figure 2. HER2 signaling pathway. HER2 as well as the other members of the EGFR family are receptor tyrosine kinases which are located on the cell membrane and responds to a wide variety of ligands. Phosphorylation of the tyrosine kinase domain in the cytoplasm initiates downstream oncogenic signaling pathways such as PI3K/AKT pathway and Ras/MAPK pathway.

c-MYC

c-MYC gene is found on chromosome 8q24 and is reported to be involved in many tumors and higher expression of this gene are associated to cause TNBC and aggressive human prostate cancer [1, 13, 14, 42]. The v-MYC, a viral oncogene is the cellular homolog of c-MYC. c-MYC is usually expressed only during cell division in order to accelerate the cell's entry into the S phase, mostly through initiation of cyclin E-CDK2 activity. The product of this gene play a crucial role and acts as a nuclear transcription factor which is responsible for the regulation of a network of genes that account for up to 15% of human genes. These genes have multiple functions which include metabolism, apoptosis, proliferation, and differentiation. Remarkably, c-MYC protein expression triggers two main functions: (1) cell proliferation as well as (2) cell apoptosis and up till now, this paradox has not been fully elucidated [1, 13, 14, 43-46].

Meta-analysis studies revealed that this gene is overexpressed three times or more in 1%–94% of BC patients (average of 15.5%), even though considerable variation in amplification of the oncogene exists. The overexpression of c-MYC usually takes place before the gene amplification, may be due to enhanced transcript or stability of the protein. Excitingly, correlation was observed in the amplification of both c-MYC of HER2 [1, 13, 14, 47].

RAS

RAS genes have been extensively investigated during last three decades. The RAS family is implicated in crucial cell signaling pathways that control cellular survival, differentiation and proliferation [17, 19]. The RAS family genes are located on three different regions of the chromosomes; the first gene that encodes HRAS is located on chromosome 11p15.5, the second one encodes 2 splicing variants, KRAS4A and KRAS4B and is on chromosome 12p12.1, the last one is located on chromosome 1p13.2 and transcribes NRAS kinase. The function of the RAS protein is phosphorylation of some secondary messengers involved in different cellular pathways related to cell differentiation, motility, proliferation and apoptosis [17, 19]. Using a modified BC cell line to express

HRAS and NRAS, it was revealed that HRAS activation of the Rac-MKK3/6-p38 pathway could be involved in BC metastasis. Moreover, results of breast tumors analysis revealed that RAS is linked to only less than 5% of cases of BC. However, RAS hyperactivity is linked to overexpression of HER2 and/or EGF. Point mutations were identified in 7 of the 40 cell lines analysed, with the majority of the mutations in KRAS [17, 19].

Estrogen and its Receptor

Estrogen is a mammalian sex hormones and is synthesized in ovaries of premenopausal females and somewhat in extragonadal (e.g., breast) tissues [20]. It is a strong mitogenic hormone and is very crucial in the development of the breast as well as it is also involved in BC. Two different genes encode 2 types of estrogen receptors (ERs); (1) ERα and (2) ERβ genes (Table 2). First one is present on chromosome 6q25.1 and second one is present on chromosome 14q22–24 [19, 20]. There is functional and structural similarity in the expressed proteins of these genes. The ERα isoform which is important in mediating cell division in breast tissues is overexpressed in the first stages of BC development. Studies have shown that almost two-third of BC tissues express higher ER levels compared to normal tissues. Estrogen binds to ERα leading to the formation of an established receptor dimer that becomes phosphorylated and exposes the DNA-binding domain and transcriptional activation domains. The role played by ERβ in BC is still yet to be elucidated [20].

Tumor Suppressor Genes

The main function of the oncogenes is cell growth and proliferation, however, the tumor suppressor genes (TSGs) work in antagonistic fashion relative to the oncogenes (**Figure 3**). The TSGs may undergo processes like physical deletion or recombination that lead to a loss of their function and subsequently cause different kinds of mutations. Because of the significant role they play in the hereditary of cancer, they are termed susceptibility genes. There are many types of TSGs with each playing a vital role in the growth of BC. Among them are PTEN, TP53, RB, and BRCA1 and BRCA2.

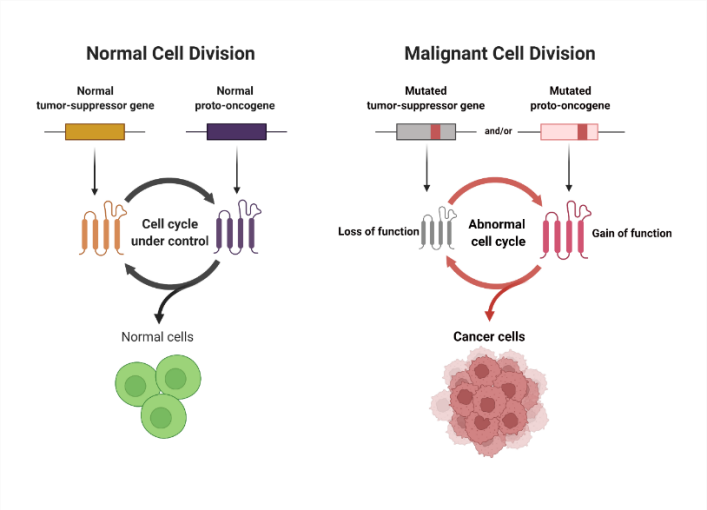

Figure 3. Drawing shows normal cell division and malignant cell division. In normal cell division, cell cycle is under control and cells are large and well differentiated with highly condensed nuclei. While in malignant cell division, after mutations, cell cycle is abnormal, cells are undifferentiated and with scanty cytoplasm.

Phophatase and Tensin Homolog (PTEN)

PTEN is a phosphatase and is present in humans and is located on chromosome 10q23.1 [21]. Mutations in PTEN is the first step in the development of different types of malignancies in humans. Mutations can cause malignancy in breast, lungs, prostate and can cause glioblastoma. The key function of PTEN is halting the cell cycle and promoting apoptosis. Other functions of the PTEN

include regulating invasion, migration, and cell adhesion, particularly through extracellular molecules such as the integrins [1, 21, 22, 48].
Loss of PTEN has been identified to be widespread in sporadic BC, although mutation of the gene is rare and methylation of the PTEN promoter has been confirmed to be liable for the inhibition of gene expression of the PTEN (Khan et al 2004). Studies have shown that PTEN is involved in the regulation of the P13K/AKT/mTOR pathway in breast carcinogenesis with new evidences indicating that the regulation involve complex set of events [1, 21, 22, 49-53].

Tumor protein P53 (TP53)
TP53 gene encodes a protein called tumor protein p53 and is present on chromosome 17p13 [1, 23]. TP53 protein encoded by the TP53 gene activates transcription and has a three domains structure similar to other activators. It carries out various functions in the cell such as apoptosis, cell cycle arrest, differentiation, DNA repair, and angiogenesis inhibition. It's core function is to maintain cell's genome integrity prior to cell division, which is the reason for its name as the "Guardian of the Genome". Mutations in p53 that cause disruption in this pathway may result in cell division even if the DNA becomes damaged [54, 55, 56]. This type of damage can instigate carcinogenesis. Similarly, mutations in TP53 pathway causes BC. A genetic or an epigenetic mutation was reported in the TP53 gene in around 20-40% of BCs, this was said to be associated with poor. The type of mutation in the TP53 has been established to be a point mutation leading to malfunctioning and non-degradable protein that results in tumor cells. High rate of mutation in TP53 has also been related with carriers of germline mutation in the BRCA1 and BRCA2 [23, 24, 57-59].

Retinoblastoma (RB)
Retinoblastoma protein that is anti-oncogene or TSG and is situated on chromosome 13q14 [25]. It binds with over 110 cellular proteins and these proteins are categorized into 3 classes. These class are: 1. Kinases, their regulators, and phosphatases, 2. transcriptional regulators and 3. miscellaneous proteins that perform different functions such as DNA replication and regulation of cell cycle [25, 60]. These proteins mainly play their roles in inhibition of cell proliferation and growth, cell differentiation, and to control cell apoptosis. In cancer patients, about 80% of RB gene cellular pathways are affected by mutations, making RB gene very crucial factor in carcinogenesis in humans. There are certain phenomenon which are responsible for the loss of activity of RB in BC and these are functional inactivation through cyclins A or E overexpression, chromosomal deletion, intragenic mutation, and transcriptional silencing such as promoter hypermethylation [25, 26, 61, 62].

BRCA1 and BRCA2
BRCA1 (BReast CAncer gene 1) and BRCA2 (BReast CAncer gene 2) (BRCA1/2) are the most important susceptibility genes for BC [27, 28]. The discovery of BRCA1/2 was a seminal event in the field of cancer, particularly in breast cancer, about 20 years ago and this event paved the way for the exploration of other high-penetrance susceptibility genes. According to reports, BRCA1/2 are diagnosed in 80% of familial BC patients and just 5%–10% of all BC cases [27, 28, 29]. These genes are recognized as high-penetrance variants, particularly in Ashkenazi Jews (one in 40 women has BRCA1/2 mutation) with as high as 90% penetrance [63-66].

The BRCA1 protein constitute part of the genome surveillance complex (BASC) made up of DNA repair and TSG proteins, like MSH2 and the RAD50-MRE11-p95 complex, whose function include recombination-mediated repair of breaks in the double-stranded DNA [13, 14, 28, 29]. BRCA 1 gene is transcribed at late G1 phase and during the S phase of the cell cycle. After the DNA sustains a damage, the regulator proteins ATM, ATR, or CHK2 that are involved in cell's tumor suppression pathways swiftly phosphorylate BRCA1 in order to an active it. The BRCA1 then halts the cell cycle at the S and G2/M checkpoints prior to the cell division. Therefore, the general function of the BRCA1 is inhibition of oncogenes and amplification of the TSGs (29). BRCA2 gene is found on chromosome

13q12 and is composed of 27 exons, out of which 26 encode for a nuclear phosphoprotein with size of 384 kDa [13, 29, 67, 68].

CHEK2

Many CHEK2 mutations have been described to be associated with higher risk of BC. The CHEK2 variants confer moderate BC risks up to (2–4-fold) and differ widely among different geographical regions and ethnic populations [2, 70, 71].

Treatment Strategies

There are two immune-based treatment strategies: passive immunotherapy and active immunotherapy. The treatment of people with HER2 via monoclonal antibodies (pertuzumab and trastuzumab) falls under passive immunotherapy category [32]. On the other hand, active immunotherapy generally refers to cancer vaccines. The aim of vaccine therapy is to stimulate or elicit type I CD4+ and CD8+ T-cell immune responses against tumour specific antigens or tumour-associated antigens [32].

Types of BC Vaccines

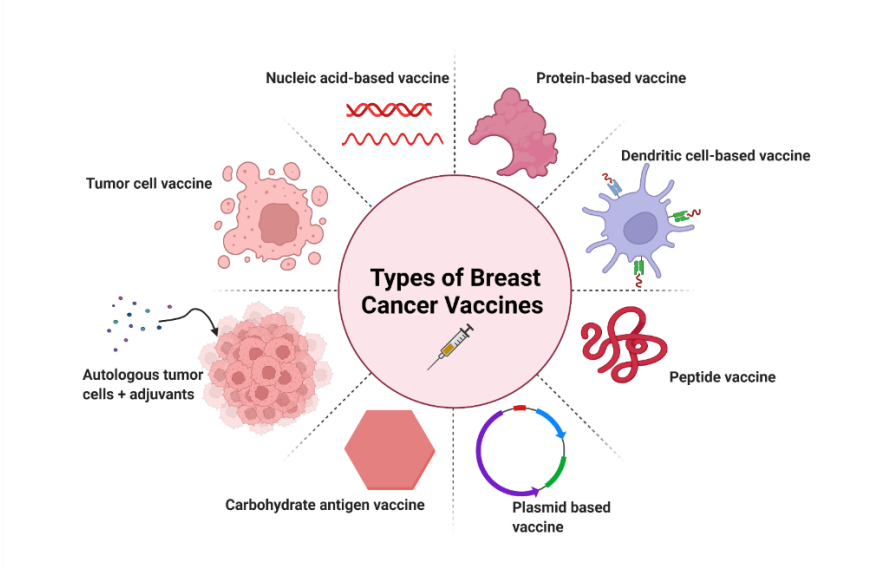

Figure 4. Different types of BC vaccines. These vaccines can be divided into the following types according to their formulations and approaches: tumor cell vaccine, nucleic acid-based vaccine, protein-based vaccine, dendritic cell-based vaccine, peptide vaccine, plasmid based vaccine, carbohydrate antigen vaccine, and autologous tumor cells + adjuvants vaccines.

Vaccination approaches involve two very important points: administration routes and optimization of vaccine regimens. There are different vaccine formulations for BC, however, recognizable targeted antigen to induce a therapeutic effect is crucial for each vaccine formulation (**Figure 4**).

Protein-Based Vaccine

This type of vaccine is developed using amino acid sequence of a shortened or a whole piece of cancer antigen protein which has larger size of amino acid sequence than peptides. Vaccine enables approval, processing, and presentation of various major histocompatibility complex class-I and -II epitopes and is not human leukocyte antigen restricted.

However, presentation process of this vaccine could be inefficient. Moreover, the body response to this vaccine is immeasurable because there are no specific markers available for protein-based vaccines [26, 32, 72-75].

Tumor Cell Vaccine (TCV)

This type of vaccine is developed by using cancer cells taken from the individual during surgery. The obtained cancerous cells are destroyed and then altered in the laboratory to increase and stimulate polyclonal T cell response. TCVs, individualized formulations developed with radiations-treated autologous malignant cells acquired from the cases, can reflect the actual antigen repertoire within the patient's tumors and present a wide range of tumor antigens in the context of self-major histocompatibility antigens. Nonetheless, there are some disadvantages of TCVs; these vaccines have endogenous normal self-antigens which can cause a potential autoimmune reaction, they show poor immunogenicity, TCVs production on large scale is time-consuming. Moreover, for vaccine production, large amount of cancerous tissues are required [26, 32, 33].

Carbohydrate Antigen Vaccine (CAV)

The antigens involved in this type of vaccine are aberrantly overexpressed by different carcinomas and it can be appealing approach toward tumor immunotherapy since these antigens act as best candidate to be integrated in a tumor vaccine (**Figure 4**). For example, antitumor capability of Sialy-Tn (STn), is expressed exclusively on the cell surface of different carcinomas, including breast cancer. Nevertheless, the poor immunogenicity of carbohydrate antigens is a major shortcoming of such vaccines. The immunogenicity could be improved by using modified-carbohydrate antigens for immunization [32, 33].

Dendritic Cell-Tumor Cell Fusion Vaccines

Dendritic cells are very potent antigen-presenting cells which are able to induce antitumor immunity by linking both types of immune systems. An ongoing effort to develop the dendritic cell-based vaccination approach is the combination of dendritic cells with tumor cells. This fusion by biological, chemical or physical ways produce heterokaryons, which comprise dendritic cell-derived major histocompatibility complex (MHC) class I, II and molecules for co-stimulation along with whole cancer-derived large collection of tumor-associated antigens (TAAs). Transfection of tumor cells is also possible with a viral fusogenic membrane glycoprotein and pelleted with DCs to attain a DC-tumor hybrid. More important, this type of cell fusion is capable to offer the entire collection of cancer antigens from the ancestral cancer cell to stimulate both pathways (MHC class-I and –II). Though, this type of vaccine is very harder to make in comparison to the DC-based vaccine pulsed with peptides [32, 33].

DNA-Based Vaccine

In this type of BC vaccine, DNA sequences encoding tumor antigens are used and these sequences are delivered with the help of vectors and plasmids. During this method, DNA sequence is integrated by APCs and translated into the cancer antigen, which will then be processed to stimulate an antigen-specific immunity. Such vaccines are uncomplicated and easy to manufacture on large scale with low storage cost. Nevertheless, the immunogenicity of DNA-based vaccines is not efficient enough because of decreased plasmids uptake ability as well as antigen expression [32, 33].

Peptide Vaccine

Peptide-based vaccine approaches are used to design customized vaccines on the basis of tumor-specific antigens by using synthetic peptides. In this method, MHC class-I restricted peptide epitopes are provided to stimulate the antigen specific immune responses against the cancer antigen and this is very frequent BC prevention strategy used. In the formulations of this vaccine, short sequences of amino acid peptides are used to manufacture cheap, simple, and comparatively stable and secure during transportation, enabling large-scale production and transportation possible. Nevertheless, the binding peptides of MHC class-I do not exhibit a strong capability to stimulate CD4+ helper T cells. Therefore, this problem can cause incomplete activation of CD8+ cytotoxic T cells and transience of immune responses. This incomplete activation of T cells can be partly prevented by using synthetic peptides that are long enough to include multiple MHC class-I and-II epitopes [32, 76-79].

DC-Based Vaccine

Dendritic cells are most potent APCs with heterogeneous population of cells. DCs are capable of activating naive T cells and they effectively take up antigens, process them and offer theses processed antigens to CD4+ and CD8+ T cells after migrating to lymph nodes. Ex vivo production of DCs having tumor antigens are utilized in DC-based vaccines or transfected to express tumor antigens. The mass production of this vaccine might be very challenging as well as demanding because of the individualized ex vivo process for the maturation of dendritic cells [32, 80].

mRNA Vaccines for Breast Cancer

mRNA vaccines have remarkable therapeutic and prophylactic potential to fight against viral diseases and cancer because of their superiorities in efficacy, safety and industrial production. Tumor vaccines are classified into 4 major categories: peptide-based vaccines, viral vector-based vaccines, immune or tumor cell-based vaccines, and nucleic acid (DNA- or RNA-) based vaccines [35, 36]. The DNA and RNA based vaccines are very promising because of numerous reasons: (1) These vaccines offer simultaneous provision of numerous antigens covering many somatic tumor mutations, target tumor-associated antigens (TAAs) or stimulating both cell-mediated and humoral immune response, enhancing the possibility of incapacitating vaccine resistance. (2) This type of vaccines may encode complete sequence of tumor antigens, and overexpression of whole cancer antigens will allow APCs to concomitantly provide diverse epitopes with both class I and II patient-specific human leukocyte antigen (HLA), consequently these are less restricted by the HLAs and are expected to trigger a stronger T cell response. Most important feature of nucleic acid vaccines is, they are non-infectious and are devoid of virus or protein-derived contaminations during manufacturing. Hence nucleic acid-based vaccines are considered well tolerated for both therapeutic and prophylactic applications [36].

Over the past decades, due to technological revolution in the field of biotechnology multiple modifications in mRNA backbone and untranslated regions were possible. These modifications made mRNA more stable, less sensitive to RNases, and highly translatable, warranting mRNA as potential vaccine candidate. In vivo delivery of mRNA has been attained efficiently by encapsulating mRNA into different types of delivery vehicles (e.g., lipid nanoparticles, peptides and polymers). To date, more than twenty mRNA-based immunotherapeutics have entered clinical trials with very promising results in cancer treatments [33, 35, 36, 37, 81].

Basic Pharmacology

mRNA is a macromolecule which is complementary to genetic sequence of DNA in the nucleus of the cell nuclei and is sent to cytoplasm where it is read by a ribosome, molecular machines that read it and mRNA is translated into proteins. This kind of vaccine can induce two different responses: CD4+ T/ CD8+ cytotoxic T cell responses and antibody/B cell mediated humoral responses, these both responses are useful for effective clearance of cancerous cells [33, 39].

In vitro transcription (IVT) method has been used for synthesizing mRNAs. In this method, a bacteriophage RNA polymerase (e.g., SP6 RNA, T3, or T7) and a linear DNA template comprising the sequence of target antigen are used for the synthesis. The IVT method prevents the use of cells and their associated regulatory hurdles, resulting cleaner, quicker and simpler, mRNA production than large-scale protein production and purification [35, 36, 37, 38]. The non-replicating IVT mRNA structure is composed of an open reading frame (ORF) region (encoding target antigen sequences), flanked by five-prime (5′) and three-prime (3′) untranslated region (UTR), and further stabilized by 7-methylgaunosine (m7G) 5′ cap and 3′ poly (A) tails respectively [35, 36, 37, 38]. However, there are some limitations for the development of mRNA vaccine: (1) most important obstacle in mRNA vaccines is quick degradation of naked mRNA by extracellular RNases, failing cellular internalization and uptake of mRNA (2) Intrinsic immunogenicity of mRNA is the secondary issue, which can stimulate downstream interferon associated cellular pathway to elicit innate immunity. Despite the fact that the immunogenicity can play its role as adjuvant-like outcome for the enhancement of

immune response, but, immunogenicity paradoxically assists mRNA degradation, hence decreasing antigen expression. Additionally, the impurities in mRNA, generated during IVT procedure, will potentially activate the innate immunity, limiting mRNA translation [35, 37, 38].

Strategies to Overcome Limitations

Cap modifications

mRNAs generated by IVT procedure usually possess a N7-methylated guanosine that is introduced to the first 5′ nucleotide via a 5′,5′-triphosphate bridge for effective translation in the eukaryotic system. This m7Gppp- is usually denoted as "Cap 0". The 5′ cap dependent translation comprise of eukaryotic translation initiation factor 4E (eIF4E) and it enables the ribosome recognition and translation initiation [35, 37]. Vaccinia capping system is commonly used in vitro post-translational capping method. This capping system is based on the Vaccinia Virus Capping Enzyme (VCE). This system comprises of two subunits called as D1 and D12 and both subunits play key roles in different activities. Essential activities such as guanylyltransferase, triphosphatase, and methyltransferase are possessed by D1 subunit and these activities are necessary for introducing a complete Cap 0 structure, whereas D12 subunit functions in activating D1 [35, 39]. VCE system offers nearly 100% capping efficiency with proper orientation, but efficient expression and purification for VCE are required for large scale capped RNA production [35, 39].

Optimization of UTRs

Untranslated regions (UTRs) can impact mRNA translation efficiency and decay by interacting with RNA binding proteins. By optimizing the 5′ UTR sequences, accuracy of translation and the stability of mRNA can be enhanced by following methods: (i) assuring the absence of start codon (AUG), and non-canonical start codons (CUG) in the 5′ UTR, as both codons may disrupt the translation of ORF, (ii) assuring the absence of highly stable secondary structures, which will prevent codon recognition and ribosome recruitment, (iii) shorter 5'UTR may be added as this kind of 5'UTR is more beneficial to mRNA translation [35, 39].

Codon optimization of ORF

Optimization of cytosine (C) and Guanine (G) content in the ORF may be applied to regulate the translation elongation rate. Uridine (U) reduction is additional codon optimization approach which can be associated to an improved GC content. Furthermore, the sequence optimization can have equal proportion of each codon present naturally in highly expressed proteins in the targeted cells or by using best pairs of codons that are commonly seen in these highly expressed proteins [35, 39]. Finally, highly stable secondary structures and hairpin loops should be circumvented in the ORF. Hence, codon optimizations in the ORF should be carefully monitored to ensure moderate translation rate and high translation accuracy [35, 39].

Poly (A) tail modification

Poly (A) tail can enhance RNA stability, decelerate the degradation of RNA exonuclease, and improve translation efficiency. A suitable length of Poly(A) is crucial. The mammalian cells contain Poly (A) tail with approximately 250 nucleotides in length, however, different cells can have different preferences. Moreover, Poly (A) binding protein (PABP) can interact with 5'cap through translational initiation factors, such as eIF4G and eIF4E, forming a close-loop to impact mRNA structure [35, 39, 40].

Conclusion

In this review, we provide an inclusive survey on the basic genetics and biological aspects of BC with multiple vaccine therapeutics including mRNA therapeutics. As we have explained, BC involves complicated environmental, genetic, and epigenetic factors in how it establishes in the patient. About <30% of patients with family history of genetic BC have an identified causative gene mutation. Majority of such cases are because of an alteration in one of the highly penetrant BC genes including

PTEN, BRCA1/2, CDH1, TP53, and STK11 and there are existing strategies that give tangible direction for the management of these cases. A small number of cases of BC are due to mutations in moderate-penetrance genes (CHEK2, ATM, BRIP1, and PALB2). A small number of low-penetrance alleles have been discovered through unconventional genetic testing approaches. Whereas these genes can contribute to risk in a polygenic fashion, most probably this is pertinent to a smaller number of cases and their identification should not be considered routine practice. We wish to draw more attention of the readers to the recommendation that naturally arises from this statistic that these tumors may be initiated by heritable mutations that happen as a consequence of the aging process and lifestyle-related risk factors, rather than inherited mutations. Evolution and continuing development in molecular technology especially in genomics and systems biology has permitted the detection and identification of bases of hereditary profile of BC and contributed to the precision medicine and treatment. We wish to encourage the further development of novel diagnostic and therapeutic measures to fully realize the best possible patient outcomes for those who suffer from BC.

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