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Breast cancer: understanding etiology, addressing molecular signaling pathways, identifying therapeutic targets and strategizing the treatment

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Abstract

Breast cancer has messed the life of a greater number of women being the most common cancer affecting them worldwide. A number of risk factors contribute the breast malignancy, however, genetic drift is accountable the most. Depending on the cell origin, invasiveness and receptors involved, breast cancer is classified into various subtypes. The accurate diagnosis of breast cancer is important as it defines the prognosis and directs the type of treatment required. A number of major signaling pathways involved in breast tumorigenesis and its development include estrogen receptors (ERs), HER2, Wnt/β-catenin, Notch, Hedgehog (Hh), PI3K and mTOR pathway. Furthermore, certain enzymes like Cyclin dependent kinases and breast tumor kinases also play a vital role in cell cycle regulation and therefore, in the development of breast neoplasms. Recent studies have also enlightened the role of non-coding RNAs in breast cancer development. This review discusses various aspects of breast cancer such as its etiology, subtypes, various signaling pathways involved, targets projected by these pathways and the current treatment options based on a few of these targets. Also, the role of different genes, enzymes and non-coding RNAs related to breast tumorigenesis and development is discussed.

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INTRODUCTION

Cancer has created great havoc globally in the past decades and is one of the major concerns of the healthcare society. Cancers could be in various types such as cervical, ovarian, breast, lungs, and cancer of the blood, prostate cancers and many others. Out of these, breast cancer is the most common female cancer worldwide, representing nearly a quarter of all cancers (Siegel *et al.*, 2019; Sung and Ferley, 2021).

The disease creates a lot of mental and emotional disturbances in patients due to major surgeries involved and greater chances of relapse or metastasis that may lead to death (Davis and Tami, 2020). However, in recent years, due to fundamental understanding of the disease at its cellular and genomic level, modern and graceful therapies for the disease are evolving. Genomics, proteomics, biomedical research, system biology and molecular biology have enabled researchers to understand the major signaling pathways involved in breast

cancer, molecular pathogenesis of the disease and heterogeneity of breast tumors. It also uncovered the most common genetic mutations involved in breast cancer, participation of non-coding RNAs and metastasis mechanism within it. Assimilation of this information has assisted scientists to progress in the novel discoveries of therapeutic targets of breast cancer. This identification of targets has made medicinal chemists to develop the chemical actives that can be used for the treatment of the disease (Feng and Spezia, 2018).

All these aspects starting from the etiology, identification of the type of breast cancer, its prognosis, and the signaling pathways involved, the therapeutic target which it epitomizes and the respective therapy, is discussed in this review. Besides, the role of key enzymes, genetic mutations involved and the part of epigenetics in the development of breast cancer is also overviewed.

Risk Factors of Breast Cancer

Genetic trends

The risk of developing breast cancer in women nearly doubles if she includes a first-degree relative diagnosed with breast cancer. In total, around 5 to 10 % of breast cancers are associated with gene mutations hereditary to a parent. The most popular form of hereditary breast cancer is inherited mutation in the BRCA1 or BRCA2 gene. Statistical analysis pointed 55 to 65 % lifetime chance of development of breast cancer in women with a BRCA1 mutation. Also, this possibility is 45% in women with a BRCA2 mutation. Also, the occurrence of one of these two mutations in women indicates the prevalence of breast cancer at a younger age as well as developing cancer in both breasts. It also increases the risk of ovarian cancer as well (Colditz *et al.*, 2012).

The inherited mutations in other genes, other than BRCA mutations, often raise the risk of breast cancer, albeit less severe and dramatic. Some of these mutated genes include CHEK2, PTEN, PALB2, TP53, CDH1 and STK11. CHEK2 gene mutation will increase the risk of breast cancer by about 2-fold. The inherited mutation of PTEN may cause Cowden syndrome followed by a higher risk of non-cancerous and cancerous tumors in several parts of the body, including the breast. Mutation in the PALB2 gene extensively increases the risk of breast cancer as the protein coded by it interacts with the protein made by BRCA2. TP53 gene inherited mutation cause Li-Fraumeni syndrome with an augmented risk of developing several types of cancer, breast cancer being the most associated. The inherited CDH1 gene mutation triggers hereditary diffuse gastric cancer with an elevated risk of invasive breast lobular cancer. Mutation in STK11 can develop to Peutz-Jeghers syndrome with a higher risk of many types of cancer, including breast cancer (Feng and Spezia, 2018).

In high-risk women, judiciously reviewed genetic testing of mutations in the BRCA1 and BRCA2 genes, as well as other less frequently mutated genes, may be helpful for the early detection and/or prevention of breast cancer (Polyak, 2007).

Non-genetic risk factors

Race and ethnicity

Incidence rates of breast cancer are highest in non-Hispanic whites followed by non-Hispanic blacks, however, the later race has a higher mortality rate than the earlier due to the concerned disease. Other races or ethnic groups such as American-Indian, Hispanic and Asians have lower incidences as well as death rate due to breast cancer (American Cancer Society, 2019).

Presence of breast lesions

Women with proliferative lesions with atypia in ducts or lobules of breast tissue present a very high risk of breast cancer. The examples of such lesions are atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia (ALH). Also, women with proliferative lesions without atypia have a small chance of a potential breast cancer diagnosis. These lesions include ductal hyperplasia, fibroadenoma, sclerosing adenosis, papillomatosis or radial scar.

The non-proliferative lesions in the breast may have a small impact on the risk of breast cancer. Such non-proliferative lesions include simple cysts, mild hyperplasia of breast tissue, adenosis, phyllodes tumor, single papilloma, duct ectasia, periductal fibrosis, squamous and apocrine metaplasia, epithelial-related calcifications, other tumors (lipoma, hamartoma, hemangioma, neurofibroma, adenomyoepithelioma), or mastitis (Dyrstad *et al.*, 2015).

Lobular carcinoma in situ (LCIS) is the breast area/s where excessive cell growth takes place in the lobules of milk-producing glands. Women diagnosed with this neoplasia have a higher-than-average chance of developing invasive breast cancer later on in life. LCIS is typically diagnosed before menopause, most commonly between the ages of 40 and 50 (American Cancer Society, 2019).

Birth control and contraceptives

The risk of breast cancer is high in women who use hormonal method for contraception like the use of birth control pills, depot formulations, implants,

injectables, intrauterine devices (IUDs), vaginal rings etc. However, this risk gets neutralized once the schedule is stopped breast (American Cancer Society, 2019).

Exposure to diethylstilbestrol (DES)

Nonsteroidal estrogen drug diethylstilbestrol (DES) is seldom used nowadays. In the past, particularly during the 1940s to 1970s, it has been used as a support medication in pregnant women with a history of recurrent miscarriage. However, follow-up studies of medication revealed some possible adverse effects, including the increased risk of breast cancer in women who had this drug treatment during their pregnancy. Moreover, "DES daughters", the women whose mothers took DES during pregnancy, may also have a slightly higher chance of developing breast cancer (Hilakivi-Clarke, 2014).

Hormone replacement therapy

Many women experience menopause associated with hot flushes, mood swings, sleep disturbances, vaginal dryness etc. All these changes are attributed to a reduced level of estrogen in the body. Therefore, physicians suggest Hormone replacement therapy (HRT) that can relieve menopause symptoms as well as can avoid osteoporosis. HRT usually consists of combination of estrogen and progesterone or only estrogen. Studies had revealed that combined HRT increases the risk of breast cancer and chances of mortality due to it. However, the risk declines and is reversible after the stoppage of therapy within a limited time. Women who have been using combined HRT for more than five years run a slightly higher risk. Short-term "only estrogen" use after menopause doesn't appear to significantly raise the risk of breast cancer till 15 years. Also, the threat of breast cancer is comparatively lower with the newer HRT agents such as Tibolone (Chlebowski and Rohan, 2015).

One of the governing factors in breast cancer risk due to HRT is the form of hormone used, too. Bioidentical or natural hormone use is neither inherently safer nor more efficient and should therefore be considered as having the same cancer risks as any other type of HRT. In terms of synthetic hormone analogues, research studies pointed that medroxyprogesterone acetate and norethisterone pose a greater risks than dydrogesterone and progesterone (Mirkin, 2018).

Radiation therapy

The risk of developing breast cancer is high in women who had undergone chest radiation therapy for another cancer at their young age while their breasts were in developing condition. However, this factor doesn't affect breast cancer risk unless the treatment has been obtained after the age of 40 (Sun and Zhao, 2017).

Age margins of menstrual cycle

Typically the women who have long-term exposure to estrogen and progesterone have a slightly higher risk of breast cancer. So the woman who starts menstruating earlier, before the age of 12, and the women whose menopause occurs after the age of 55, experiences longer 'estrogen and progesterone effects,' increasing their risk of breast cancer (American Cancer Society, 2019)).

Pregnancy age and breastfeeding

Women who do not have a child or who have their first kid after age 30 have an increased risk for breast cancer that is pointedly greater. Additionally, women with multiple pregnancies or who had been pregnant at an early age have a lesser risk of breast cancer. However, a study showed pregnancy increases the risk of triple-negative breast cancer (Nichols and Schoemaker, 2019).

Breastfeeding to children directly reduces the risk of breast cancer in mothers, specifically if it is carried out for 1.5 to 2 years. Possibly this relationship is based on the fact that nursing decreases the number of menstrual cycles in women's life.

Excessive alcohol consumption

This factor is specifically related to an increased risk of breast cancer, and this factor's risk increases with alcohol intake. Studies have also shown that in women who have two to three drinks a day, the risk of breast cancer rises by 20 percent relative to women who do not take it (American Cancer Society, 2019).

Lack of physical activity

Now a day, much evidence pointed out that after menopause, physical activity in women decreases the danger of breast cancer, though; the exact mechanism for this connection is not established. This may be due to the fact that physical activity regulates the hormonal and energy balance of the body and holds it there.

Obesity

Most of the estrogen in the female body is produced by ovaries before menopause, and a small amount of it is also produced by fatty tissues. Nevertheless, ovaries stop producing estrogen, which makes fatty tissues the main source after menopause. Consequently, obese women produce more estrogen levels due to higher fatty tissue content, which leads to an increased risk of breast cancer. However,

the connection between obesity and the danger of breast cancer has yet to be completely evident.

Other than these major risk factors, other factors that also contribute to the development of breast neoplasm are tobacco smoking, exposure to different environmental chemicals and pollutants, as well as night shifts in the working environment (American Cancer Society, 2019).

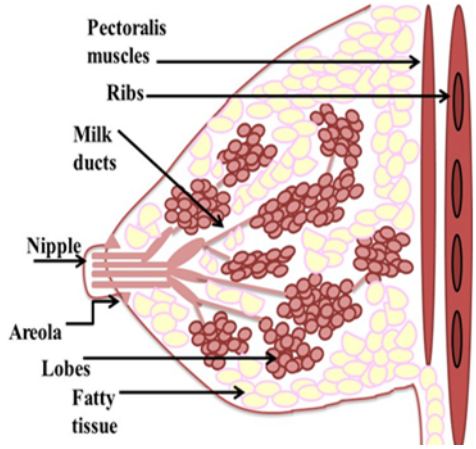


Figure 1: Anatomy of breast

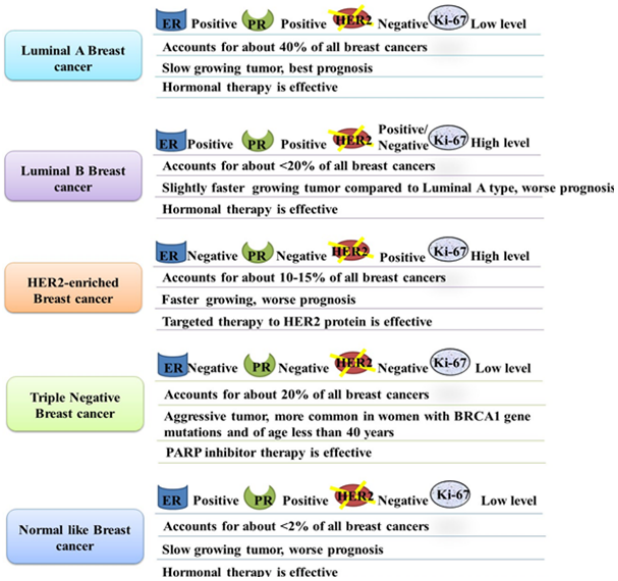


Figure 2: Intrinsic types of breast cancer with their description

Classification of Breast Cancer

Anatomy of Breast

Medically, the breasts are known as the mammary glands. They are located on top of the chest wall muscles, pectoralis major and minor. The mammary glands consist of specialized milk producing glandular tissues and fatty tissues. The milk-producing part of the breast is divided into 15 to 20 sections, called lobes. Again, there are smaller structures

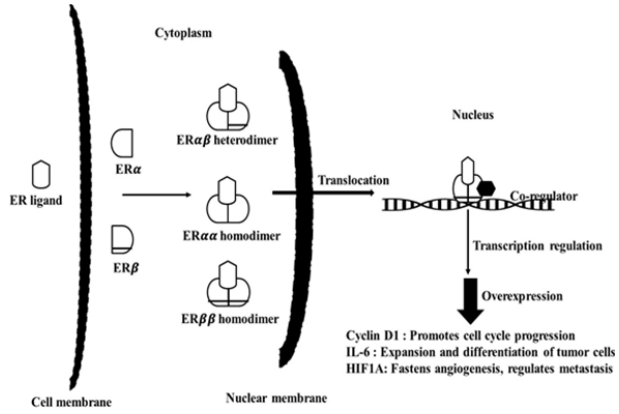


Figure 3: Estrogen receptor signaling pathway

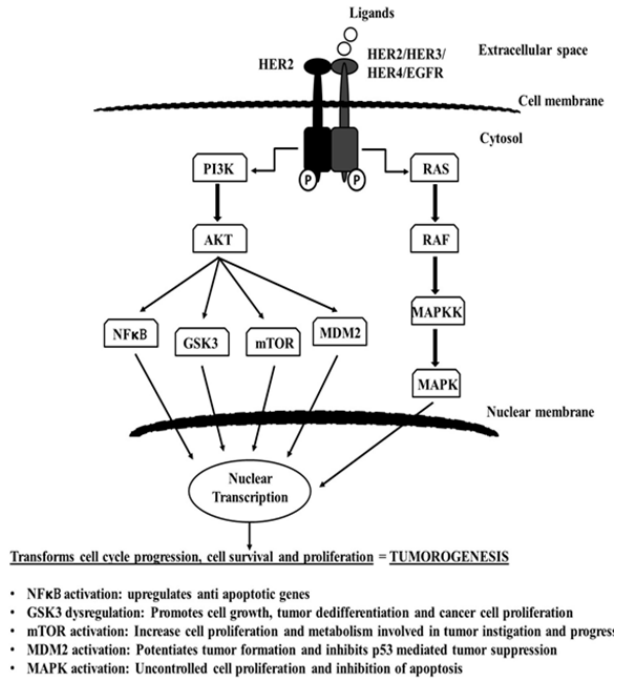
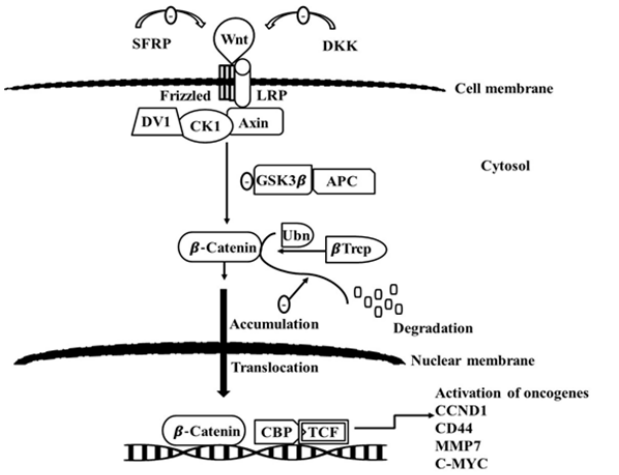


Figure 4: HER2 pathway showing the downstream tumorigenic signaling pathways that are PI3K/AKT pathway and Ras/MAPK pathway

within each lobe, called lobules, where milk is produced after stimulation. The produced milk travels through ducts, which are interconnected to form a network system. These ducts unite to form larger ducts that end in the nipple, leaving skin. The dark skin area around the nipple is called the areola. In between lobes and ducts, the breast includes fat tissue, connective tissue and ligament of Cooper. Together, they give shape to the breast. The breast anatomy is shown in Figure 1.

Blood vessels and lymphatic vessels are located throughout the breast tissue that supply blood to breast tissue and drains the excess fluid from it, respectively. The drained fluid is transported by lymphatic vessels to the lymph nodes located in



- CCND1: Codes for Cyclin D1 and alters cell cycle progression
- CD44: Regulates metastasis of cancer cells
- MMP7: Regulates invasion and metastasis of cancer cells
- C-MYC: Proto-oncogenes involved in cancer metabolism, also hinder some tumor suppressor genes

Figure 5: Canonical Wnt/β-catenin signaling pathway

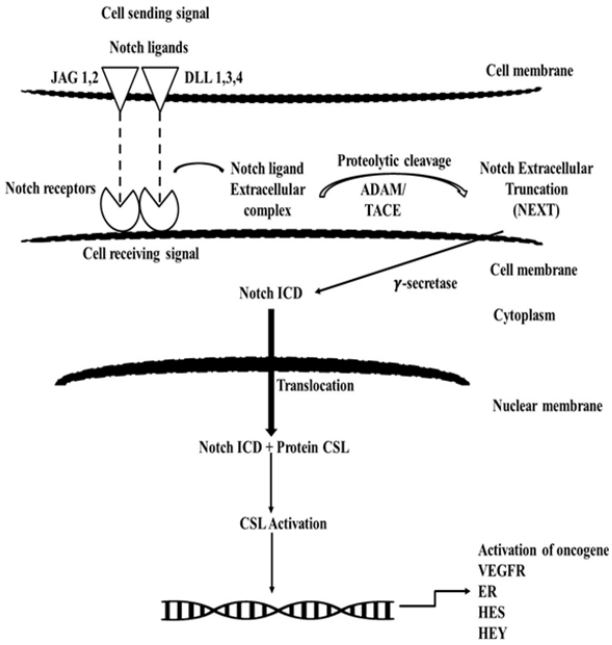


Figure 6: Notch signaling pathway

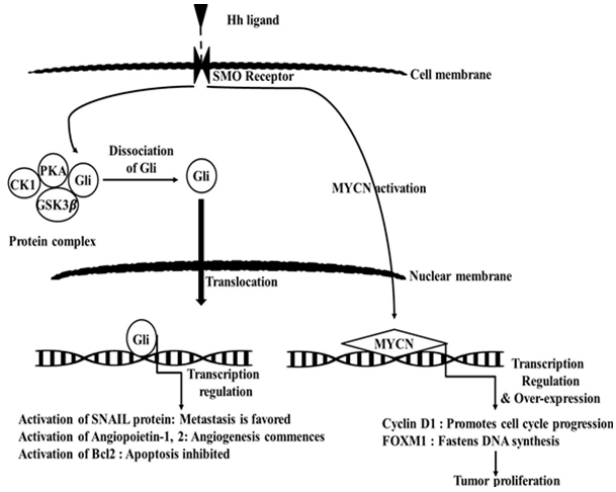


Figure 7: Hh signaling pathway

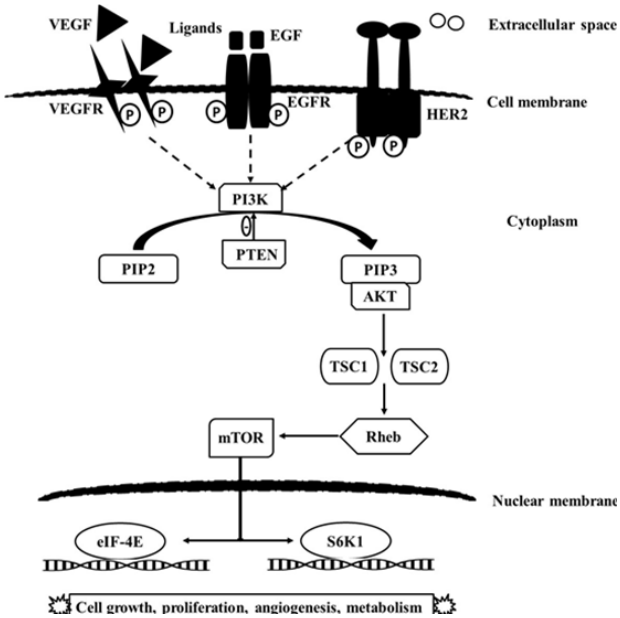


Figure 8: The PIK3CA pathway

the axilla (underarm area) and behind the sternum. Nerves provide sensation to the breast (Gaskin, 2017).

Breast cancer classification based on cell origin

The Breast cancer is divided into two major classes depending on cell origin, carcinoma and sarcoma. Carcinomas type breast cancer originates from the epithelial portion of the breast, which consists of the cells lining lobules and the terminal ducts responsible for milk production. It is the most common type. Sarcomas of breast cancer are rare (<1%), originating from blood vessel cells and myofibroblasts, the stromal portion of the breast (Allison, 2012).

Breast cancer classification based on invasiveness

Carcinoma type breast cancer is further classified based on invasiveness and the corresponding pathological features as non-invasive (or in situ), invasive (infiltrating), and metastatic breast cancers (Allison, 2012).

Non-invasive (or in situ) breast cancer

These cancers remain located inside the milk vessels or lobules in the breast. However, they have a high potential to transform into invasive form. Therefore, non-invasive cancers are also referred to as pre-cancers.

Ductal carcinoma in situ (abbreviated as DCIS; Intraductal carcinoma)

This pre-invasive type of breast cancer develops inside the normal ducts. Though it is not invasive, it has a high tendency to develop into invasive forms, hence appropriate, timely treatment is necessary to avoid the transformation.

Invasive (infiltrating) breast cancer

These cancer cells invade and spread beyond the normal breast ducts and lobules and expand into the surrounding stromal tissue of the breast. These type of cancers also pose a high risk to spread to other areas of the body, which typically includes lymph nodes. Sometimes, they even affect the other body organs and can be nominated for metastatic breast cancer. Invasive cancers are more prevalent in older women of age 55 or more.

Invasive breast cancer is classified into Invasive Ductal Carcinoma (IDC) and Invasive Lobular Carcinoma (ILC), depending on the cell and tissue type involved. With about 80% of all breast cancers constituting invasive ductal carcinomas, IDC is the most common form of breast cancer. It is further sub classified into several subtypes as tubular carcinoma of the breast, medullary carcinoma of the breast, mucinous carcinoma of the breast, papillary carcinoma of the breast, and cribriform carcinoma of the breast. Invasive Lobular Carcinoma accounts for around 10-15 percent of all breast cancers, standing as the second most common type of breast cancers. Although women at any age can be affected by ILC, it is more common in older women. Both IDC and ILC have characteristic genetic abrasions, molecular arrangements and pathological structures. Therefore, they have different prognosis and treatment and can be distinguished accurately by expert medical professionals (Allison, 2012; Feng and Spezia, 2018) .

Metastatic breast cancer (Stage IV or Advanced breast cancer)

This type of breast cancer is not confined to breast tissue but spreads and magnifies to the other organs in the body. The metastasis of breast cancer is commonly found in lymph nodes of the armpit and lungs. The liver, brain and bones also get affected by these cancer cells in the late stages of life. This type of cancer even tends to return and spread attributed to residual microscopic tumor cells or micrometastases following initial treatment or surgical removal of the primary tumor. The reappearance of breast cancer and its metastasis is observed in about 30% of the women diagnosed with early-stage breast cancer (Giuliano *et al.*, 2018).

Molecular subtypes of Breast cancer

Due to the recent advances in gene expression profiling, breast cancer can be classified into five intrinsic subtypes depending on the expression of estrogen receptor, progesterone receptor, human epidermal growth factor 2 receptors (HER2) and the basal cluster, a distinctive cluster of genes. All these types of cancer differ crucially from one another in their prognosis as well as in the therapeutics (Vidal *et al.*, 2016). This molecular or intrinsic classification of breast cancer is summarized and differentiated in Figure 2.

Major Signaling Pathways in Breast Cancer

Development and Progression

A number of complicated signaling pathways in the human body control cell creation, multiplication, proliferation, existence and migration. This normal cell communication via different mediators of pathways is interrupted by cancer cells that ultimately results in uncontrolled cell proliferation and migration. Eventually, this dysregulation is the result of genetic alterations and epigenetic modifications that causes malignancy. Mutation of proto-oncogenes causes the hyperstimulation of cell development signaling pathways or inactivates the tumor suppression pathways by suppressing the vital negative regulators of signaling. Estrogen receptor (ER) signaling, HER2 signaling and canonical Wnt signaling are the major signaling pathways that regulate normal mammary gland development and meddling in their working results in breast malignancy (Juliano, 2020). This review will emphasize such principle signaling pathways that are responsible for breast cancer.

Estrogen Receptor signaling and ER-Positive breast cancer

Two classes of estrogen receptors (ERs) exists as nuclear estrogen receptors (ERα, ER$_\beta$) and membrane estrogen receptors which are mostly G protein-coupled receptors (ER-X, GPER, Gq-mER). The former estrogen receptors are the main transcription factors that regulate the expression of target genes on ligand binding. In addition to DNA binding, they perform other non-transcriptional functions like post-translational modifications of many enzymes and proteins (Xue and Zhang, 2019; Chi and Singhala, 2019).

ERα and ER$_\beta$ are coded by two different genes, ESR1 and ESR2, respectively and have significant sequence homology. Both these receptors have six functional domains and have an ability to form a homodimer (ER$\alpha\alpha$/ ER$_{\beta\beta}$) as well as a heterodimer (ER$\alpha\ \beta$) on the binding of ligand. These receptor dimers then translocate for transcription control in the cell nucleus from the cytoplasm. The DNA binding domain of ER dimer bind with estrogen

response elements (ERE) region of target genes and utilizes the co-regulators for directing of gene transcription. Rather than this ERE-dependent pathway, ERs also act as co-regulators for other transcription factors and, in turn, controls the target gene expression. Pathogenesis of breast cancer reflects the major contribution of ERα in breast cancer development as about 75% of these diseased cases show their higher expression. BRCA1 inhibits the ERα signaling and acts as a tumor suppressor (Girgert *et al.*, 2019).

A number of studies confirmed that ERs by interacting with Cyclin D1 promotes breast cancer cell growth. The cyclin D1 is one of the main activator of cyclin dependent kinase 4 and 6 (CDK4, 6), which are responsible for switching the cell cycle from the G1 to the S phase in many cancer cells. Thus, ERα fastens the tumor cell growth rate (Manavathi *et al.*, 2013). The estrogen receptor pathway is summarized in Figure 3.

Therefore, in ER positive breast cancer, estrogen antagonist therapy provides greater benefits. This therapy includes the use of drugs like Tamoxifen, Raloxifene and Fulvestrant (Sharma *et al.*, 2018). They function by preventing the process of transcription by facilitating the binding of co-repressors to ERE. Also, the use of selective CDK4 and 6 inhibitors has shown a new ray for a breast cancer patient as they suppress G1 to S phase transition in the tumor cell. Examples of the drugs belonging to this category are Palbociclib, Ribociclib and Abemuciclib. The synergistic effects were observed with the combined therapy of these selective CDK4/6 inhibitors and antiestrogen therapy (Finn *et al.*, 2016).

A number of studies were carried out to establish the role of ERα in breast cancer development and in the last decade, studies are directed to predict the role of ER$_\beta$ in the same. Different in vitro and in vivo studies have confirmed that the progression of breast cancer is associated with reduced expression of ER$_\beta$, pointing to it as a breast cancer suppressor. The p53 knockout mouse model suggested the breast cancer suppression activity attributed to its interaction with p53, a tumor suppressor protein. However, further studies need to be carried out to establish its definitive role and mechanism in breast cancer pathogenesis (Girgert *et al.*, 2019).

HER2 signaling and HER2-Positive breast cancer

Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor family and is a type of tyrosine kinase receptors. It is present in many normal tissues. Being a kind of tyrosine kinase receptors, it has an extracellular ligand-binding site, transmembrane lipophilic section and an intracellular domain with tyrosine kinase activity. Though the receptor has no identified ligand, on binding with its family members HER3, HER4 or EGFR, HER2 receptors undergo dimerization followed by autophosphorylation of tyrosine residues of the intracellular domain and activates a number of cellular signaling pathways (Iqbal and Iqbal, 2014). The principal pathways activated by this mechanism are the mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3-kinase (PI3K) pathway. These pathways are chiefly involved in cellular proliferation, survival and differentiation (Sever *et al.*, 2015).

In 15–30% of cases of invasive breast cancers, HER2 gene amplification takes place, resulting in HER2 overexpression. This results in triggering; over and prolonged signaling of MAPK and PI3K pathways causing excessive cell growth, survival, differentiation, angiogenesis and invasion. Dimerization of HER2 is also responsible for p27 protein mislocalization and degradation, affecting the development of the cell cycle. Other membrane receptors such as Estrogen receptor, Insulin-like growth factor receptor 1 are also found to activate HER2 receptor and consequent signaling (Iqbal and Iqbal, 2014). The HER2 activated signaling pathways can be visualized in Figure 4.

The progression of HER2 positive breast cancer can be prevented by targeted therapies that block major components involved in HER2 signaling, including the HER2 receptor itself. Trastuzumab (Herceptin) is an anti-HER2 antibody that subdues HER2 signaling by destabilizing its heterodimer or by causing its lysosomal degradation. Also, it elicits an antibody-mediated immune response. Pertuzumab is one more monoclonal anti-HER2 antibody that interferes with HER2 signaling chiefly by inhibiting HER2-HER3 heterodimer formation. Both these antibodies bind to the extracellular domain of the HER2 receptor and elicit their action. Another class of HER2 therapy includes the use of Tyrosine kinase inhibitors (TKI) that binds to the intracellular domain of HER2 and prevent HER2 signaling. Lapatinib and Neratinib are well-known TKI that acts by this mechanism. One of the most efficacious Anti-HER2 antibody-drug conjugate is Trastuzumab-emtansine, also known as T-DM1 or Kadcyla. It delivers the drug conjugate at target and consists of a mitotic poison Maytansine (DM1) conjugated with Trastuzumab (Larionov, 2018).

Canonical Wnt/β -catenin signaling in breast cancer

This pathway is instigated by the binding of Wnt pro-

teins to both co-receptors Frizzled and low-density lipoprotein receptor-related protein 5 and 6 (LRP). These Wnt proteins are lipid-modified, secreted glycoproteins that facilitate cell-to-cell communication. Cell growth, cell differentiation and cell death are regulated by them. On the interaction of Wnt proteins with receptors, different cytosolic proteins as Axin, CK1 and DV1 are rolled to the cell membrane, which promotes glycogen synthase kinase (GSK)-3β protein inhibition. The GSK3β protein is the negative regulator of the Wnt pathway and its inhibition encourages the Wnt mediated signaling. Also, in the absence of Wnt proteins, GSK3β proteins phosphorylate β-catenin which make it detectable for E3 ubiquitin ligase β-Trcp that promotes proteosomal degradation of β-catenin. However, as the GSK3β proteins are inhibited in Wnt signaling, the degradation of β-catenin is prevented, leading to its accumulation in the cytosol, followed by its translocation in the nucleus. The β-catenin in the nucleus co-transcripts and activates the CBP and TCF transcription factor, which leads to activation of oncogenes such as c-MYC, MMP7, CD44, CCND1 and others. The Wnt/b-catenin pathway was remarkably activated in basal-like breast tumors, and its nuclear localization is correlated with a worse prognosis. Wnt signaling inhibitors like DKKs and SFRPs act as tumor suppressors by leading to β-catenin degradation, thus hampering the transcription of related oncogenes (MacDonald *et al.*, 2009). Wnt signaling pathway can be visualized in Figure 5.

Wnt signaling is potentially triggered constitutively in breast cancer by an autocrine mechanism. The role of the concerned pathway in breast cancer can be evidenced by studies that have shown higher levels of β-catenin in about 50% of clinical breast cancer patients (Zhan *et al.*, 2017). Also, Dv1, the positive regulator of Wnt signaling, is amplified in 50% of breast cancers. And in consistence with these findings, FRP1, Frizzeled related protein 1, a secreted inhibitor of the pathway is lost in 78% of breast malignancies and is also linked with poor prognosis. In addition to this, the APC expression is lost in around 45% of breast cancer cases (Feng and Spezia, 2018).

This pathway can be targeted by the use of Porcupine inhibitors, WntFZD antagonists, LRP5/6 inhibitors and DVL inhibitors. Porcupine is the instigator for the secretion of various Wnt ligands. So, the inhibitors of Porcupine can halt the initiation of the pathway. Several active chemical moieties are in the stages of a clinical trial to prove the safety and efficacy of these agents. WntFZD antagonists like Ipafricept, is a recombinant fusion protein, compete with Wnt ligands to bind with FZD receptors. The monoclonal antibodies like Vantictumab (OMP-18R5) are also studied extensively that targets Frizzeled proteins. LRP5/6 inhibitors like BMD4503-2, a quinoxaline moiety, are also being developed and studied for their activity as a down-regulator of the Wnt pathway. Similarly, DVL inhibitors are being developed and investigated to understand their potential to use them as pathway inhibitor (Zhang and Wang, 2020).

The Notch signaling pathway

This pathway is involved in the regulation of pivotal cellular progressions as well as mediating cell-to-cell communication. The Notch signaling mainly deals with the Triple negative breast cancer (TNBC) initiation, development as well as progression. Notch 1 and Notch 4 receptors are specifically overexpressed in TNBC. There are five notch ligands identified to date as Delta-like ligands (DLL) 1,3,4 and Jagged (JAG) 1, 2. On binding of these ligands to Notch receptors present on an adjacent cell, a Notch ligand-receptor complex is formed, cleaved by ADAM/TACE induced proteases forming Notch extracellular truncation (NEXT). γ-secretase act on it, forming Notch intracellular domain (ICD), which is then translocated into the nucleus from the cytoplasm. In the nucleus, Notch ICD binds with transcription factor CSL, Lag-1 and suppressor of hairless Su(H), leading to CSL activation that further leads to activation and overexpression of different genes such as VEGFR3, ER, Hes and Hey. These genes govern the cell cycle progress, angiogenesis and apoptosis (O'toole and Beith, 2013). This signaling pathway is outlined in Figure 6.

The key component involved in notch signaling is responsible for the alteration of transcription factors in Notch ICD. It has been produced from NEXT by the action of enzyme γ-secretase. So if the enzyme is inhibited, other downstream processes can be automatically inhibited and TNBC progression can be prevented. Therefore, research is going on for the development of γ-secretase inhibitors that can prevent oncogenesis and cancer progression by this pathway (Dumbrava, 2018).

Hedgehog (Hh) signaling pathway

It is a highly coordinated and ordered pathway associated with the initiation, development and spread of tumors in different kinds of cancer. There are three distinct gene homologues for this signaling path, as Sonic Hh (Shh), Desert Hh and Indian Hh; however, the Shh pathway is the most involved gene homologue in malignancies. On binding of Hh ligand, the pathway triggers, causing activation of Smoothened (SMO) protein, a seven transmembrane protein. This stimulated SMO protein causes

dissociation of a transcription factor, Gli, from a large protein complex and facilitates its nuclear translocation. In the nucleus, the activated Glia targeted genes are modified and partake in transcription that results in alteration of the dynamic between transcriptional factors and proteins, consequential in TNBC growth. This transcription also raises the SNAIL protein and angiopoietin-1, 2 that are responsible for metastasis and angiogenesis.

In addition to this mechanism, one more mode is involved in tumorigenesis by the Hh pathway, which includes instigation of MYCN gene by SMO that increases cyclin D and FOXM 1. As discussed earlier, Cyclin D is involved in the faster development of tumor by hastening the cell cycle progression. Similarly, FOXM1 is involved in harmonizing the DNA synthesis by regulating the genes associated with cell-cycle progression. Thus, it can be stated that the development and progression of TNBC are triggered by the activation of MYCN. Figure 7 depicts the involvement of the Hh pathway in the development of TNBC (Jamdade *et al.*, 2015; Carballo and Honorato, 2018).

The Hh signaling pathway can be blocked by a steroidal alkaloid isolated from Veratrum californicum, Cyclopamine, an SMO antagonist. Their pharmacokinetic studies are carried out in rodents, dogs and cynomolgus, which proved its 33% oral bioavailability and a half-life of 4 hours. So, a number of derivatives of Cyclopamine are being studied for their pharmacological action on the Hh pathway as well as for their improved pharmacokinetic characters. Thus, the SMO protein antagonist class can prove an efficient treatment for TNBC (Jamdade *et al.*, 2015; Chen, 2002).

The PI3K Pathway

This pathway is known as one of the key protein synthesis regulatory signaling pathways and is involved in cell growth, differentiation, and migration. It is initiated by various exogenous growth factors like EGF, VEGF, different hormones and nutrients. After binding of these ligands to tyrosine kinase type membrane receptors, phosphorylation of tyrosine residues take place leading to its activation. Consequently, there is the activation of PI3K, phosphatidylinositol 3-kinase which causes the conversion of PIP2 (3,4-bisphosphate phosphatidylinositol) to PIP3 (3,4,5-triphosphate phosphatidylinositol) by phosphorylation. This PIP3 then binds with AKT (also known as protein kinase B, PKB) with the support of PDK1 and PDK2 (phosphatidylinositol-dependent kinase 1 and 2), leading to activation of Rheb (a small GTPase and a Ras-homolog usually enriched in the brain) by inhibiting the TSC-1 and TSC-2 dimerization. Activation of Rheb is essential for mTOR stimulation. Under normal situations, TSC-1 and TSC-2 exit as a dimer which functions as an inhibitor of Rheb, in turn preventing the activation of mTOR. AKT phosphorylates TSC-2 and avoids TSC-1 and TSC-2 complex formation, consequently; stimulating mTOR.

The S6K1 (Ribosomal protein p70S6K) and eIF-4E binding protein are the two major downstream targets of mTOR. mTOR starts and promotes protein synthesis by interacting with these two factors. This whole pathway is summarized in Figure 8 . Around 18% of breast cancer patients report irregular activation of this pathway due to gene mutation.

In the PI3K/AKT/mTOR signaling pathway, PTEN, a tumor suppressor gene, acts as an important regulatory factor. PTEN encodes a protein, PIP3 phosphatase, that dephosphorylates PIP3 and generates PIP2, prohibiting further downstream path. Research on PTEN indicated its absence or low expression in almost 33% of breast cancer patients. Luminal A or Luminal B breast cancer report 28-44% downregulation of PTEN, while 22-67% reduced expression of PTEN is found in subtypes of HER2 + and basal breast cancer (Zhang *et al.*, 2013).

PIK3CA gene mutation is also responsible for the anomalous triggering of the PI3K protein pathway. This mutation results in constant stimulation of AKT via kinase active gene products. Subsequently, breast cancer development is seen as abnormal mammary epithelial cell proliferation, differentiation and apoptotic resistance. Studies have shown that PIK3CA gene mutations occur in 20%-25% of breast cancer patients, and ER positive breast cancer is mainly correlated with the risk of this mutation (Gonzalez-Angulo and Ferrer-Lozano, 2011).

In vitro studies and animal models have shown that PTEN deficient or PIK3CA mutated tumors are more vulnerable to PI3K pathway inhibition. One such API, Everolimus, also known as Rapamycin, has been approved to treat advanced breast cancer (Janku, 2017).

The mTOR pathway

Insulin-like growth factor 1 (IGF-1R) receptors, members of the fibroblast growth factor receptor (FGFR) family, the epidermal growth factor receptor (EGFR/ERBB) and others are the key upstream positive regulatory factors for mTOR. Studies have pointed to the higher expression of IGF-1R and FGFR in breast cancer. Also, as discussed above, Rheb is an mTOR direct upstream regulator and increased levels of Rheb mRNA are present in many breast tumors. The mTOR negative regulators include

PTEN, PI3K and TSC (tuberous sclerosis complex). Their levels are depleted, or expression has been found significantly reduced in many breast cancer subtypes. Basically, mTOR is a catalytic subunit consisting of mTORC1 and mTORC2, two distinct complexes. mTOR complex 1 (mTORC1) consists of mTOR, Raptor, GβL (mLST8), and Deptor, while mTOR complex 2 (mTORC2) consists of mTOR, Rictor, GβL, Sin1, PRR5/ Protor-1 and Deptor. mTORC1, in its activated state, phosphorylates its two major downstream targets, i.e. p70 S6 kinase and 4E-BP1, promoting genetic transcription and mRNA translation, subsequently increasing protein synthesis. It also favors ribosome biogenesis, adipogenesis and causes suppression of autophagy via ULK1 and Atg13. mTORC2 regulates cytoskeletal dynamics by triggering PKC and retains the flow of nutrients and ions and thus cellular growth through SGK1.

Combinations of mTOR inhibitors are being studied to effectively treat multiple breast cancers, such as HER2-positive and TNBC. One such combination, Everolimus and Lapatinib, is in clinical trial phase II. In addition, clinical trials are being performed for the triple combination of Temsirolimus, Cisplatin and Erlotinib (Jamdade *et al.*, 2015).

Role of Enzymes in Development of Breast Cancer

Role of cyclin dependent kinases (CDKs) and Breast tumor kinase (BRKs) in breast cancer

Cyclins, CDKs, and CDK inhibitors govern the progression of the cell cycle. As the neoplasms represent uncontrolled cell proliferation, they need to trigger the cell cycle to grow continuously. Several studies have shown that positive cases of breast cancer overexpress cyclin D1 and cyclin E while expression of CDKI p27Kip1 is reduced. It has also been found that over-expression of both cyclin D1 and HER2 is associated with decreased recurrence-free survival in breast cancer positive patients and tamoxifen responsiveness in the same (Sutherland and Musgrove, 2004).

Palbocyclib, the oral CDK4/6 inhibitor, prevents the advancement of the cell cycle and has been approved for hormone receptor-positive, HER2-negative metastatic breast cancer as a combination with Fulvestrant (Walker and Wedam, 2016).

Breast tumor kinase (BRK) is also overexpressed in about 60% of breast cancer cases. This non-receptor tyrosine kinase is involved in breast cancer cell proliferation and migration. Activation of this enzyme increases MAPK activity and cell proliferation as well as migration in breast cancer. Similarly, depletion of BRK ruins the EGFR signaling as well as cellular migration (Miah *et al.*, 2012).

Cancer Gene Mutations in Breast Cancer

BRCA1/2 mutations in breast cancer

The name "breast cancer susceptibility genes 1 or 2" (BRC1/2) indicates that it plays a role in breast cancer development. As stated in the etiology section, almost 10%-20% of breast cancer patients have at least one first-degree relative who has been affected by the disease and mutation in the BRC1/2 is present in up to 20% of these women with a family history of breast cancer. The occurrence of BRCA mutations is relatively more among women as well as men of Ashkenazi Jewish ethnicity. Furthermore, the risk of this mutation is also high in women with ovarian cancer. A minor risk of developing breast cancer is also observed in the BRCA-mutation carrier women with a smoking tendency.

A number of studies have indicated that the most common form of BRCA gene mutation is a frameshift mutation, which causes premature stop codons to be produced, lowering the levels of mature RNAs and functional proteins. The proteins encoded by BRCA1/2 genes are involved in the DNA repair mechanism and therefore contributes to tumor suppression. As a result, mutations in the BRCA genes decrease DNA repair capacity and raise the risk of breast cancer by five to six times (Mehrgou and Akouchekian, 2016; Narod and Salmena, 2011).

Other gene mutations in breast cancer

Though a BRCA gene mutation is linked to an increased risk of breast cancer, it is not the only gene that can influence cancer risk. Other gene mutations that contribute to elevating the risk of breast cancer include PI3K, ATM, TP53, PTEN, LKB7, PALB2, CHEK2 and CDH1 gene. The products of these genes are mainly involved in DNA repair, cell cycle, an apoptosis regulation. Mutation of these genes alters the functionality and operation of the cell cycle, causing its dysregulation and consequently increasing uncontrolled cell growth (Feng and Spezia, 2018).

Role of Non-Coding RNAs in Breast Cancer

Complete and accurate transcriptomic studies revealed that up to 80% of the human genome is non-coding, while only 2% of it encodes proteins. These non-coding RNAs (ncRNAs) mainly controls gene expression and other biotic activities. Depending on the size, structure and regulatory activities that ncRNAs control, they are classified as long noncoding RNAs (lncRNAs, >200 nucleotides), microRNAs (miRNAs, <200 nucleotides), small nucleolar RNAs (snoRNAs), and piwi RNAs (or

piRNAs).

A number of studies carried out to reveal the role of these ncRNAs in tumorigenesis and their development indicated abnormal manifestation of about 215 lncRNAs in breast cancer patients. These lncRNAs, along with other protein-coding genes, are involved in activating PI3K, EGFR and TGF-β pathway and, therefore, tumorigenesis. Furthermore, it is found to be one of the key regulator in the mammary gland development process and therefore, its dysregulation may cause the appearance of malignancy in breast tissue. It is also speculated that lncRNAs are also involved in the regulation of the S-phase. Hence, significant efforts are carried out to identify the best suitable biomarker involved in S-phase associated with lncRNAs that can predict tumorigenesis.

Additionally, in the quest of finding novel possible therapeutic targets of TNBC, MANCR, mitotically-associated noncoding RNA, a type of lncRNA was studied and is currently under a greater investigation for its proposed association with TNBC.

The miRNAs are vigorously studied and characterized for their role in tumorigenesis. Similarly, aberrant methylation in a number of miRNA genes is associated with oncogenesis throwing light on their role as probable tumor suppressors genes or oncogenes. A number of studies have also evidenced the fact that lncRNAs, as well as other noncoding RNAs like miRNAs, impacts both the co- and post-transcriptional gene regulation in association with RNA-binding proteins (RBPs).

Thus, the drivers for mutations of ncRNAs, their sequence and their relationship with other cell cycle regulating factors need to be explored significantly so that one can reach the novel approach to diagnose and treat the malignancy (Meseure *et al.*, 2015).

CONCLUSIONS

This review discusses genetic, cellular and molecular aspects of breast cancer. From this discussion, it can be concluded that while genetic factors contribute the most to breast cancer origination, however, other factors like inherited BRCA1/2 mutations and lifestyle of the person also affects to a major extent. The molecular subtypes of breast cancer differ substantially in the therapeutic targets they present and, therefore, the possible therapeutic options used to treat them. More therapeutic targets can be also be identified if the signaling pathways involved in cell cycle regulation are studied in-depth. Advancement in genomics and proteomics study has also enabled scientists to recognize different diagnostic biomarkers, especially for metastatic breast cancer. Therefore, if suitable biomarkers are identified, then suitable measure can be taken by the patients to stop the appearance or progress of the disease. Also, if the reliable therapeutic targets are further identified, then it can be very much beneficial to breast cancer patients as the therapy based on it can be developed, which will improve the efficacy while minimizing the associated side effects.

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