Chapter 6

Endocrine and Cell Surface Receptor Signaling in Breast Carcinogenesis

Ibrahim O. Alanazi and Zahid Khan

Additional information is available at the end of the chapter

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Abstract

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer-related death in female. To better understand the growth and progression as well as therapeutic management, breast cancers are grouped based on histopathological and molecular classification. A number of factors have been implicated in the development of breast cancer. Various cell surface as well as hormone receptor signaling play crucial role in breast cancer initiation and progression. This chapter briefly discusses few of the important receptor signaling pathways and the various strategies in practice as well as at different stages of development to target these pathways.

Keywords: breast cancer, estrogen receptor, HER1, HER2, tyrosine kinase inhibitors, WNT signaling, therapeutic monoclonal antibodies

1. Introduction

Cancer is considered to be the leading cause of death in developed countries and the second in developing countries. The burden of cancer is growing in economically developing countries due to population aging and adaption of cancer-associated lifestyle including smoking and Western diet. In 2008, it has been estimated that around 12.7 million cancer cases and 7.6 million cancer deaths have happened. Of these about 56% of the cases and 64% of the deaths have been recorded in the economically developing countries [1].

Breast cancer in female is the most common diagnosed cancers and the leading cause of cancer-related death in developed and developing countries. It accounted for 1.38 million

new cancer cases (23%) and 458,400 (14%) of the total cancer deaths in 2008 worldwide. Of these, about 50% of the cases of breast cancer and 60% of breast cancer deaths takes place in developing countries including Western and Northern Europe, North America, Australia and New Zealand [2]. In the United States, in 2008, the American Cancer Society (ACS) estimates that almost 182,500 women were diagnosed with breast cancer and during the year about 40,500 women lost their life due to breast cancer.

The breast is made up of different types of tissues including fatty, lymphatic and connective tissue. A female breast is organized into 15–20 sections called lobes. Each of these lobes contains many smaller glandular structures called lobules which responsible for milk production. The lobes and lobules are connected through a network of tubes called ducts through which milk flows and reaches the nipple [3].

Majority of breast cancers arise from the cells in the duct or from milk-producing cells in the lobules. Increase in the incidence of breast cancer observed in epidemiological studies is as a result of breast cancer risk factors. About 20–30% of diagnosed cancer cases may be associated with these factors and their activity that lead to deregulation of the normal cellular processes into neoplastic transformation of breast cells [4]. However, about 75–80% of women with breast cancer have no identifiable risk factor [4]. Therefore, different system or model is required to be utilized to examine this cancer.

2. Risk factors of breast cancer

Being a women and getting older are the main influence to have breast cancer. It has been reported that women with age 30has a lower chance (about 1 in 1500) of developing breast cancer compared to women with age 40 (about 1 in 173) [5]. Thus, being 40 years of age or above poses significant risk for developing breast malignancy. There are several other factors that play crucial role in developing breast cancer, such as history of cancer in first-degree relatives, history of mammary gland diseases, early menarche, late menopause, Caucasian race, and late childbearing after 35 years of age. Causes of breast cancer development have also been linked to lifestyle-related factors including alcohol consumption, not being physically active, being overweight or obese and using hormone replacement therapy. Risk of having breast cancer may result from combination for these factors as reviewed by Singletary [6] as can be seen in **Table 1**.

Breast cancer classification systems have been utilized in order to organize the heterogeneity of this disease. Over many decades, these systems have been developed in order to assist in prognosis and treatment. The breast cancer classification models evolved due to advances in cancer research and understanding of the molecular heterogeneity of breast cancers. These classifications are based on histological and molecular variations in breast cancer subtypes [7]. Such classifications assist in understanding the growth and progression of breast cancer as well as in their therapeutic management.

Risk factor	Category at risk	Comparison category	Relative risk
Alcohol intake	2 drinks per day	Non-drinker	1.2
Body mass index	80th percentile, age 55 or greater	20th percentile	1.2
Hormone replacement therapy with estrogen and progesterone23	Current user for at least 5 years	Never used	1.3
Radiation exposure	Repeated fluoroscopy	No exposure	1.6
	Radiation therapy for Hodgkin's disease	No exposure	5.2
Early menarche	Younger than 12 years	Older than 15 years	1.3
Late menopause	Older than 55 years	Younger than 45	1.2–1.5
Age at first childbirth	Nulliparous or first child after 30	First child before 20	1.7–1.9
Current age	65 or older	Less than 65	5.8
Past history of breast cancer	Invasive breast carcinoma	No history of invasive breast carcinoma	6.8
Other histologic findings	Lobular carcinoma in situ	No abnormality detected	16.4
	Ductal carcinoma in situ	No abnormality detected	17.3
Breast biopsy	Hyperplasia without atypia*	No hyperplasia	1.9
	Hyperplasia with atypia	No hyperplasia	5.3
	Hyperplasia with atypia and positive family history	No hyperplasia, negative family history	11
Cytology (fine-needle aspiration, nipple aspiration fluid)	Proliferation without atypia*	No abnormality detected	2.5
	Proliferation with atypia	No abnormality detected	4.9–5
	Proliferation with atypia and positive family history	No abnormality detected	18.1
Family history	1st-degree relative 50 years or older with postmenopausal breast cancer	No 1st- or 2nd-degree relative with breast cancer	1.8
	1st-degree relative with premenopausal breast cancer	No 1st- or 2nd-degree relative with breast cancer	3.3
	2nd-degree relative with breast cancer	No 1st- or 2nd-degree relative with breast cancer	1.5
	Two 1st-degree relatives with breast cancer	No 1st- or 2nd-degree relative with breast cancer	3.6
Germline mutation	Heterozygous for BRCA1, age < 40	Not heterozygous for BRCA1, age < 40	200
	Heterozygous for BRCA1, age 60–69	Not heterozygous for BRAC1, age 60–69	15

*There is controversy over whether pathologic hyperplasia detected in breast biopsy samples is directly equivalent to cytologic hyperplasia detected in samples obtained through FNA or nipple aspiration.

Table 1. Risk factors for breast cancer.

3. Classification of breast cancer

3.1. Histopathological classification

Breast cancer can be broadly classified based on the location and aggressiveness of the disease. Two main classes of breast cancer are in situ carcinoma and invasive (infiltrating) carcinoma. Breast carcinoma in situ can be divided into two types. Ductal carcinoma in situ (DCIS) originates from the cells lining the ducts that transport milk to the nipple while lobular carcinoma in situ (LCIS) occurs in the cells of lobules, the milk-producing glands at the end of breast ducts [3, 7]. Both DCIS and LCIS are premalignant lesions that do not invade deeper or spread through the body. Women with these lesions have higher likelihood of getting cured but also have increased risk of developing invasive breast cancer in the future. DCIS is significantly more common accounting for 80–90%, while LCIS accounts for 10–20% of breast cancer cases [3]. DCIS has been traditionally sub-classified to five well-recognized subtypes as Solid, Papillary, Micropapillary, Cribriform and Comedo based on the architectural features of the tumor [8]. Invasive carcinomas, similar to in situ carcinomas are differentiated into histological subtypes. These subtypes include infiltrating

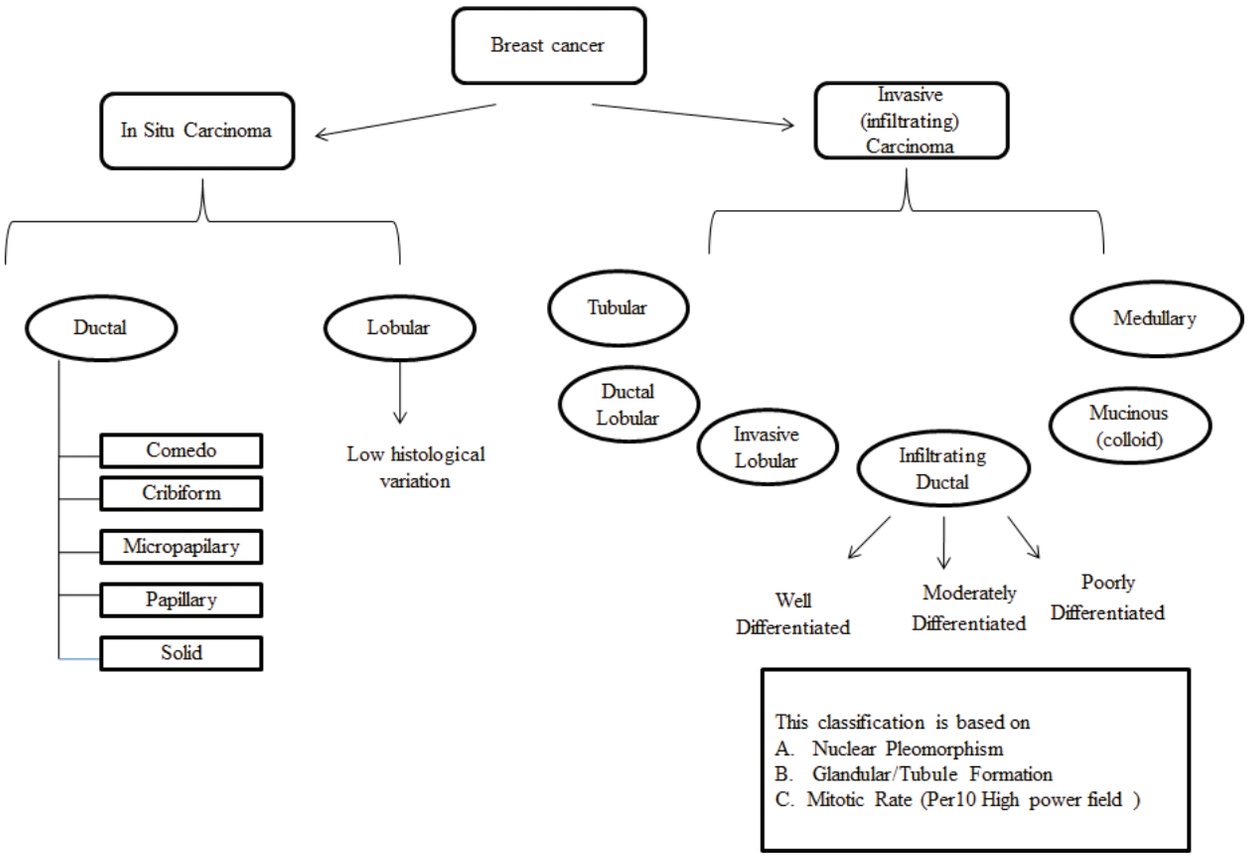

Figure 1. Histological classification of breast cancer subtypes. Modified from Malhotra et al., 2010 [7].

ductal (IDC), invasive lobular (ILC), mucinous (colloid), medullary, tubular and papillary carcinomas (**Figure 1**) [7]. IDC is considered as the most common histological subtype of breast cancer, and it accounts for 70–80% of all invasive lesions [9], while ILC is the second most prevalent and accounts for roughly 10% of all breast cancers. These subtypes differ from each other based on clinicopathologic aspects, natural history, epidemiology and molecular alterations [10].

3.2. Molecular classification

Breast cancer is a heterogeneous disease with diverse histological and molecular variations determining the biological behavior and therapeutic response. The occurrence as well as death due to breast cancer is on the rise globally despite advances in the development of diagnostic techniques and medications. There are many factors including age, family history, receptor status and others that have been investigated to assess patients' risk and treatment selection. It has been proven that receptor status is the most valuable in determining prognosis and responsiveness to therapy [11, 12]. Based on the receptor status, breast cancers are divided into three main groups. The first group includes estrogen receptor (ER) or progesterone receptor (PR) positive, while the second group comprises tumors that tested positive for human epidermal growth factor receptor 2 (HER2) with or without ER and PR positivity. Finally, triple-negative breast cancer (TNBC) is defined by the absence of ER/PR expression and HER2 amplification [11]. Targeted therapy is available for breast cancer patients that express ER, PR or HER2 receptors; however, no standard treatment options are in practice for TNBC patients. Traditional chemotherapeutic regimens are utilized for this type of patients [13].

A number of techniques including immunohistochemistry (IHC), DNA microarray technology, fluorescent in situ hybridization (FISH) are utilized to reveal molecular differences within the same or different histopathological specimens [14–16]. Using IHC and DNA microarrays lead to the identification of five discrete subtypes of breast cancer. These subtypes include luminal A (ER^+ and/or PR^+ and $HER2^-$), luminal B (ER^+ and/or $PR^+$and $HER2^+$), HER2 overexpressing (ER^- and PR^-, $HER2^+$), basal-like ($ER^-/PR^-/HER2^-$, cytokeratin $5^+/6^+$ and/or epidermal growth factor receptor $(EGFR)^+$) and normal breast-like. These different breast cancer subtypes are diverse in prognosis and therapeutic management [11]. Microarray classification of breast cancer is represented in **Table 2**. This classification is based on two types of epithelial cells including luminal and basal cells (and/or myoepithelial) in human mammary gland. These cells can be identified using IHC technique as luminal cells express ER and PR receptors and keratins $8^+/18^+$, whereas basal cells are keratins $5^+/6^+$ and 17^+ [17, 18].On the other hand, TNBC can be identified by ER^-, PR^- and $HER2^-$ using IHC range between 0 and 1 or by FISH negative if 2+ on IHC [15].

Various receptors including estrogen receptor (ER) and other growth factor receptor signaling pathways play an important role in breast cancer initiation and progression. Targeting these receptors by specific inhibitors may lead to the inhibition of tumor growth [20].

Subtypes	ER, PgR, Her 2 Status	Other IHC features	Cell of origin	Other characteristics
Luminal A	ER + or PgR + or both, Her 2-	Keratin 8/18 + ve	Luminal epithelial cell	Younger age Best prognosis Low rates of recurrence Higher survival rate
Luminal B	ER + or PgR + or both, Her 2+	Keratin 8/18 + ve	Luminal epithelial cell	Higher tumor grade Poorer prognosis
Basal-like	ER-, PgR-, Her2−/+	Keratin 5/6/17 + ve EGFR + ve	Basal/myoepithelial cell/ Bipotent progenitor	15% Younger age Associated with hereditary BRCA 1 Poorer prognosis compared to other types Spread to axillary nodes, less common to bones
Her 2+	ER-, PgR-, Her2+	—	Late luminal progenitor	20–25% Poorer grade Lymph nodes positive Early distant metastases Poor prognosis Frequent relapse
Normal breast-like	Tumors that do not fill into any of these categories	—	Luminal epithelial cell	6–10% of all breast cancers Small tumors Good prognosis More common in postmenopausal Associated with fibroadenomas
Claudin low	ER-, PR-, Her2-	Mesenchymal markers	Stem cell	5–10% of all tumors Typically triple negative Low expression of cell-cell junction proteins (like E-Cadherin) Lymphocytic infiltrates

IHC, immunohistochemistry; ER, estrogen receptor; PgR, progesterone receptor, +, positive, − negative.

Table 2. Microarray classification of breast cancer [19].

4. Breast cancer receptors (ER, PR and HER2) and their involvement in cancer progression

4.1. Estrogen receptor (ER)

Despite the fact that a large number of potentially valuable factors have been identified, only three receptors, the estrogen receptor-alpha (ERα), the progesterone receptor (PgR), and the HER2 are utilized in clinical practice, and their assessment is obligatory [21]. Approximately 70% of all breast cancers, which belong to the molecular subtypes luminal A or luminal B, express ERα. There is strong evidence demonstrating that estrogen plays an important role in the progression and development of breast cancers, although the causes behind these malignancies still remains uncertain [22]. ER alpha positive breast cancers depend on estrogen signaling for proliferation. Binding of estrogen to ERs leads to dimerization of the receptor which then translocates to the nucleus and binds estrogen response elements in the DNA sequence. This leads to cell proliferation as a result of stimulation of target genes [23]. ERα mediates a number of molecular signaling such as mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways which are involved in cell growth and proliferation [24] as can be seen in **Figure 2**.

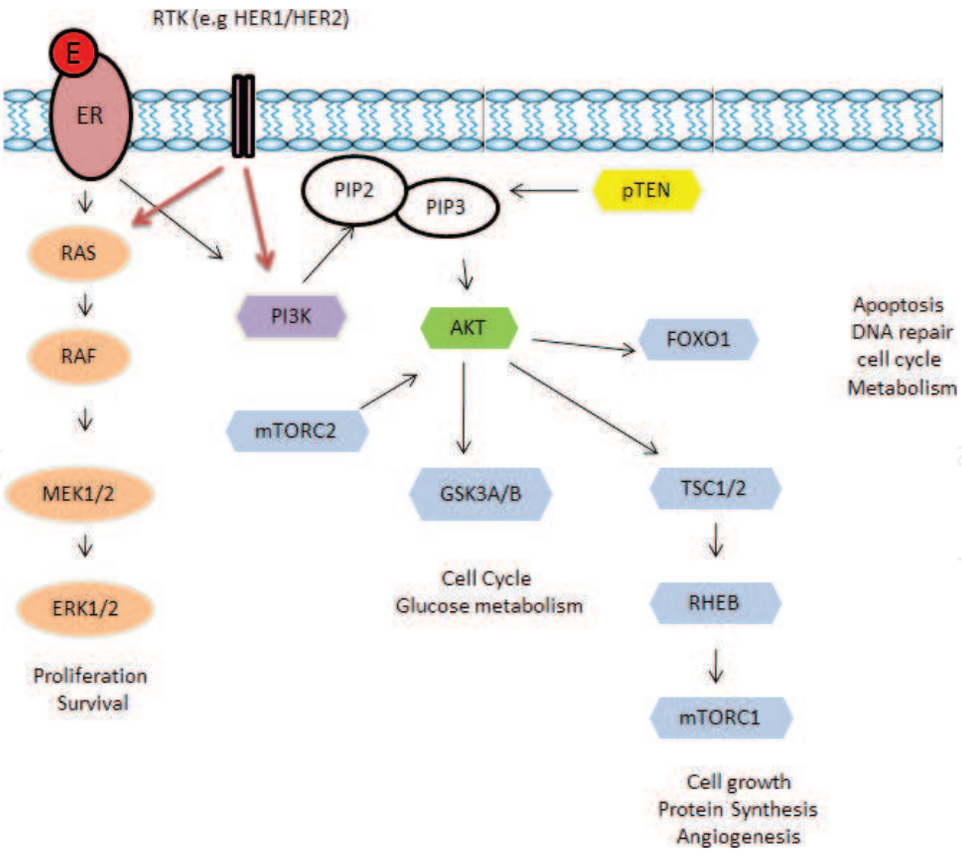

Figure 2. The PI3K/AKT/mTOR and the RAS/RAF/MEK/MAPK pathways. Modified from Toss and Cristofanilli [25].

ERα-positive breast cancer depends on these signaling for proliferation. Therefore, the most effective approach to terminate or slow the growth of this type of cancer is by blocking estrogen action in the tumor using hormone therapies. For the past few decades, one of the most widely used drug for the treatment of breast cancer is tamoxifen, which is a selective ER modulator and acts as anantagonizer for ERα function. It has been utilized as a long-term adjuvant therapy and as preventative agent in a lot of women at increased risk for the disease. Also, fulvestrant, which acts as an anti-estrogen, downregulates ERα and has been approved for clinical use [22].

Another class of breast cancer treatment drugs that have evolved are called aromatase inhibitors (AIs). These inhibitors include anastrozole, letrozole and exemestane as detailed in **Table 3**. AIs are able to inhibit the aromatase enzyme (a cytochrome P450 heme-containing protein), which is required for estrogen synthesis [26] During menopause, the level of estrogen decreases due to cessation of estrogen production by the ovaries. Hence, locally synthesized estrogen via breast adipose tissue plays crucial role in the survival and growth of ERα-positive breast tumors [27].Unfortunately, majority of patients treated with endocrine therapy develop resistance. This leads to the progression of disease and fatality. Several signal transduction pathways such as MAPK and PI3K are involved in tamoxifen resistance. These pathways are activated by growth factors including human epidermal growth factor receptor 2 (HER2). It has been noticed that MCF-7/HER2–18 tamoxifen-resistant model system that overexpress HER2 shows increased growth when cells are treated with tamoxifen. Several studies indicate that there exists a molecular cross-talk between the ER and HER2 pathways [28, 29]. Also, the mechanisms associated with AIs resistance share similarities with tamoxifen resistance, particularly in the upregulation of growth factor pathway such as HER2 and its dimerization partner epidermal growth factor receptor (EGFR/HER1) [30].

Retinoblastoma (RB) is the tumor suppressor protein that plays an important role in regulating the progression of cell cycle. This occurs by the RB inhibitory action on E2Fs which are a family of transcription factors that are crucial for the expression of S-phase genes. It has been noticed that deregulation in the RB pathway occurs in various cancers including breast cancer 2. About 50% of breast cancers overexpress cyclin D1 that lead to an aberrant phosphorylation of RB facilitating cell cycle progression [31]. Adjuvant tamoxifen-treated ER positive breast cancer patients having functional RB pathway have fewer breast cancer recurrences, while those with RB non-functional tumors have no benefit of tamoxifen. Therefore, knowing the RB status in breast cancer can be utilized as predictive factor to identify patients who will benefit from tamoxifen therapy [32]. Further, it has been observed that histone demethylase retinoblastoma-binding protein 2 (RBP2) has a potential to develop endocrine therapy resistance in breast cancer. Choi et al. demonstrated that tamoxifen resistance in vitro and in vivo occurs as a result of RBP2 overexpression, while knocking down RBP2 imparted tamoxifen sensitivity. The cooperation between RBP2 and ER coactivators and corepressors regulates a number of tamoxifen resistance-associated genes. Moreover, RBP2 increased IGF1R-HER2 cross-talk that lead to PI3K-AKT activation through demethylase activity-independent HER2 protein stabilization. Therefore, RBP2-mediated tamoxifen resistance might be overcome using combinational treatment with PI3K inhibitor and tamoxifen. ER-IGF1R-HER2 signaling cascade can be activated in various ways by RBP2 to induce tamoxifen resistance, thus making RBP2

Drug	Target	Indications/trials
Tamoxifen	ER	Treatment of metastatic estrogen receptor positive breast cancer.
		Adjuvant treatment of node-positive breast cancer in postmenopausal women following total mastectomy or segmental mastectomy.
		Adjuvant treatment of axillary node-negative breast cancer in women following total mastectomy or segmental mastectomy.
Fulvestrant	ER	Hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer in postmenopausal women who have not been previously treated with endocrine therapy.
		HR-positive advanced breast cancer in postmenopausal women whose disease has progressed after endocrine therapy
		HR-positive, HER2-negative advanced breast cancer or breast cancer that has spread to other parts of the body (metastatic), in combination with palbociclib or abemaciclib in women whose disease has progressed after endocrine therapy.
Anastrozole	Aromatase enzyme	Adjuvant treatment (treatment following surgery with or without radiation) of postmenopausal women with hormone receptor positive early breast cancer approved for the initial treatment of postmenopausal women with hormone receptor positive or hormone receptor-unknown locally advanced or metastatic breast cancer and for the treatment of postmenopausal women with advanced breast cancer that has progressed following treatment with tamoxifen.
Letrozole	Aromatase enzyme	Adjuvant treatment of postmenopausal women with estrogen receptor positive early breast cancer.
Exemestane	Aromatase enzyme	Adjuvant treatment of postmenopausal women with estrogen receptor positive early breast cancer who have received 2–3 years of tamoxifen and are switched to exemestane for completion of a total of five consecutive years of adjuvant hormonal therapy.
Trastuzumab	HER2	Treatment of patients with HER2-overexpressing breast cancer.
Pertuzumab	HER2	Used in combination with trastuzumab and chemotherapy as adjuvant treatment of patients with HER2-positive early breast cancer at high risk of recurrence.
T-DM1	HER2	Used for the treatment of patients with metastatic HER2-positive breast cancer.
Cetuximab	HER1	Pre-clinical and clinical studies especially in combination therapy to treat triple-negative breast cancer.
Panitumumab	HER1	Phase II study of Panitumumab, Nab-paclitaxel, and Carboplatin for patients with primary inflammatory breast cancer (IBC) without HER2 overexpression.
Erlotinib	HER1	Phase I study of Erlotinib and Metformin in triple-negative breast cancer.
		Pre-clinical studies in triple-negative breast cancer.
Lapatinib	HER2/HER1	Treatment of postmenopausal women with hormone receptor positive metastatic breast cancer that overexpresses the HER2 receptor and for whom hormonal therapy is indicated.
Neratinib	HER2/HER1	Adjuvant treatment of adult patients with early stage HER2-overexpressed/amplified breast cancer, to follow adjuvant trastuzumab-based therapy.

Drug	Target	Indications/trials
RO4929097	γ-secretase inhibitor (NOTCH Pathway)	Pre-clinical and early clinical trial.
MRK-003	γ-secretase inhibitor (NOTCH Pathway)	Pre-clinical studies in animal models
MK-0752	γ-secretase inhibitor (NOTCH Pathway)	A pilot study in combination with tamoxifen or letrozole in patients with early stage breast cancer prior to surgery.
PF-03084014	γ-secretase inhibitor (NOTCH Pathway)	Pre-clinical studies and phase 2 trial in patients with advanced triple-negative breast cancer with or without genomic alterations in notch receptors
Salinomycin	LRP6 (Wnt/β-catenin pathway)	Pre-clinical studies in animal models.
OMP-18R5	FZD7 (Wnt/β-catenin pathway)	A phase 1b dose escalation in combination with paclitaxel in patients with locally recurrent or metastatic breast cancer.
OMP-54F28	FZD8 (Wnt/β-catenin pathway)	Phase I study in patients with advanced solid tumors.
OTSA101	FZD10 (Wnt/β-catenin pathway)	Yttrium 90-radiolabeled OTSA101 in phase I trial in patients with relapsed or refractory non-resectable synovial sarcomas

Table 3. Approved and investigational drugs targeting estrogen receptor and other signaling pathway components.

as a potential therapeutic target for ER-driven cancer [33]. Despite these findings the mechanisms of endocrine therapy resistance are poorly understood making it a major challenge in the clinical management of this disease.

4.2. Progesterone receptor (PR)

Human breast cancers rely on estrogen and/or progesterone hormones for growth, and this effect is mediated through ERs and PRs. ER or PR positive tumors represent up to two thirds of invasive breast cancers in women whose age are less than 50 years, while about 80% of tumors in women with age above 50 years are ER positive [34]. ERα regulates the expression of PR. Therefore, the detection of PR normally indicates that the estrogen-ERα pathway is intact and functional. Once PR is expressed, the hormone progesterone activates PR leading to the upregulation of several crucial cellular function such as proliferation contributing to breast cancer growth [21]. One of the most important parameter in breast cancer management is determining the response of tumor to hormone therapy as not all patients benefit from these therapies. Patients with breast cancer overexpressing ERs and PRs are more likely to respond to hormone therapy, while tumors negative for these receptors are unlikely to get benefit from them and respond better to chemotherapy [35]. Hormone therapy provides better quality of living and improves survival. Higher expression level of PR is associated with better hormone therapy response, increased survival rate and longer time for treatment failure. It has been noticed that PR^+ is correlated with higher hormone therapy response rate independent of ER

despite the fact that the expression levels of ER and PR are correlated. Tumors with ER^+/PR^+ have higher response rate compared to ER^+/PR^-tumors. Thus, the status of PR provides essential information about how tumors will respond to hormone therapies [35].

4.3. Human epidermal growth factor receptor 2 (HER2)

Transmembrane receptor tyrosine kinases family (RTKs) consists of HER1 (epidermal growth factor receptor [EGFR]), HER2, HER3 and HER4. These receptors are involved in regulating a range of cellular processes that controls cell growth, differentiation, survival and migration. HER2 gene overexpression has been reported in 20–30% of patients with breast cancer [16, 36]. HER receptors are located at the plasma membrane and get activated by ligand binding to the extracellular domain. This binding induces the formation of homodimers or heterodimers [36]. While HER2 does not have a known ligand, HER3 is kinase-inactive and thus both these receptors signal by heterodimerization. Dimerization of these receptors leads to the phosphorylation of tyrosine residues within the receptor intracellular tyrosine kinase domain (cytoplasmic domain). These residues work as docking sites for adaptor proteins containing Src homology 2 and phosphotyrosine binding domains (PTB). These proteins activate a large number of signal transduction molecules such as stress-activated protein kinase and signal transducer and activator of transcription (STATs) and protein kinase B (PKB or AKT) and subsequent stimulation of downstream signaling pathways including STAT, PI3K and MAPK pathways. These signaling pathways are able to activate a wide range of cellular responses such as survival, proliferation, cell motility and differentiation [16]. Overexpression of HER2 and HER1 is responsible for poor clinical prognosis including unfavorable response to endocrine therapy in breast cancer patients.

4.4. HER1 and HER2 as therapeutic targets

There are two types of therapeutic strategies that have been utilized against breast cancers overexpressing HER1 and HER2 receptors. The first includes monoclonal antibodies (MAbs) that bind to the extracellular domain of the receptor interfering with the binding of endogenous ligands that activates these receptors. In the second strategy, small molecule inhibitors (tyrosine kinase inhibitors [TKIs]) bind to the tyrosine kinase domain and inhibit its kinase activity and subsequent downstream signaling (**Figure 3** and **Table 3**) [16].

Anti-HER2 antibodies have been successfully utilized in the treatment of various cancers overexpressing HER2 including breast cancer. These antibodies target the extracellular domain of HER2 and prevent receptor activation. Several monoclonal antibodies have been used including trastuzumab and Pertuzumab. They bind to extracellular domain of HER2 in order to suppress its activity by preventing receptor dimerization and subsequent phosphorylation of the tyrosine kinase domain. As a result, the initiation of downstream signaling pathways gets precluded [37].In addition, a number of TKIs such as gefitinib, lapatinib and neratinib that are approved for treatment of breast cancers binds to HER1 and HER2 tyrosine kinase domains and inhibits their downstream signaling pathways [38]. A major clinical challenge in the treatment of HER2-positive breast cancer is due to resistance to the HER2-targeted antibody trastuzumab. Aghazadeh and Yazdanparast indicated that the over-activation of signal

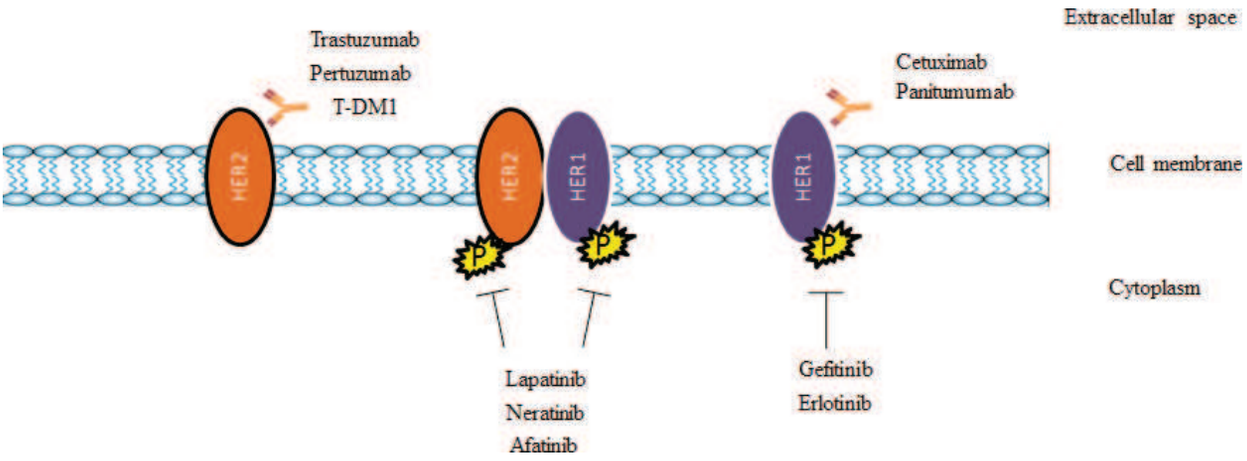

Figure 3. TKIs and MAbs target HERs for the treatment of breast, lung and several other types of cancer. Modified from Alanazi and Khan [16].

transducer and activator of transcription protein 3 (STAT3) has been associated with trastuzumab resistance. This suggests that STAT3 acts as a prognostic indicator of trastuzumab resistance in primary HER2-positive breast cancer [39]. More than 50% of breast cancer patients with aggressive and chemotherapeutic resistant condition have been detected with the phosphorylation of STAT3. Abnormal STAT3 activation due to alterations in HER2, HER1, BRCA1, and ER leads to deregulation of cell proliferation, migration, survival and angiogenesis. Several up-regulated genes including hypoxia inducible factor 1 alpha (HIF-1α) as a result of unexpected STAT3 activation have been implicated in trastuzumab resistance. During their study, Aghazadeh and Yazdanparast found that HIF-1α is an essential signaling element required to downregulate phosphatase and tensin homolog (PTEN) via Hes Family BHLH Transcription Factor 1 (HES-1) repressor during the induction of trastuzumab resistance [39]. Similarly, Sonnenblick et al. provide convincing evidence for a link between phosphorylation of STAT3 and trastuzumab resistance in HER2 positive primary breast cancers. Patients with activated STAT3 may suggest novel approaches to block the STAT3 pathway in combination with trastuzumab treatment particularly in PTEN-deleted breast cancer [40]. Additionally, it has been observed that inhibition of IL6-STAT3 pathway in PTEN-deleted HER2 positive breast cancer leads to decrease in the population of cancer stem cells and inhibits the development of distance metastasis using IL6R antibody alone or in combination with trastuzumab [41].

5. Other receptors involved in breast cancer

5.1. NOTCH signaling pathway

Notch signaling pathway within the breast cancer field attracted a huge number of research scientists over the past decade. It has been observed that Notch signaling is aberrantly activated in breast cancer and affects a number of cellular processes such as apoptosis, proliferation and cancer stem cell activity. The Notch signaling pathway consists of four receptors (Notch1, Notch2, Notch3 and Notch4) and five Delta/Serrate/LAG-2 (DSL) ligands Jagged1,

Jagged2, Delta-like1 (Dll1, Dll3 and Dll4). The activation of Notch signaling pathway occurs by the interaction of DSL ligands with Notch receptors on adjacent cells. This interaction induces a proteolytic cleavage of the Notch protein at the S2 cleavage site mediated by two enzymes, Disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) and Disintegrin and metalloproteinase domain-containing protein 17 (ADAM17). After this cleavage, the remaining part of the Notch protein will be cleaved by the γ-secretase enzyme complex and releases the Notch intracellular domain (NICD). Finally, NICD translocates to the nucleus and forms a complex with DNA-binding protein recombination signal binding protein for immunoglobulin Kappa J region (RBPj) and a member of the mastermind-like (MAML) family transcriptional coactivators (**Figure 4**). This complex activates various target genes of Notch pathway that are associated with tumorigenesis [42], cell growth, differentiation, and cell survival [43].

A number of studies indicate that aberrant activation of Notch signaling has been observed in breast cancer. The high expression of Notch signaling pathway components including Dll1, Dll3 and Dll4, Jagged1–2 and Notch receptors has been observed in invasive breast cancer. This expression is associated with poor prognosis in breast cancer patients [43, 44]. Due to the important role of Notch signaling pathway in breast cancer, it becomes a very attractive therapeutic target. At present, several classes of Notch inhibitors have been developed and are under various clinical trials including γ-secretase inhibitor (GSI), monoclonal antibodies against Notch receptors or ligands and small interfering RNA (siRNA).GSI effectively represses cancer stem cells (CSCs) in *in vitro* studies and triggers apoptosis via inhibiting proteasome activity and enhancing endoplasmic reticulum (ER) stress in tumor cells [45]. Several GSIs have completed pre-clinical and early clinical trials including RO4929097 which significantly sensitizes putative breast cancer stem cells to ionizing radiation and may be effective in treating inflammatory breast cancer (IBC), MRK-003 in combination with trastuzumab induced tumor regression and prevented tumor recurrence post-trastuzumab

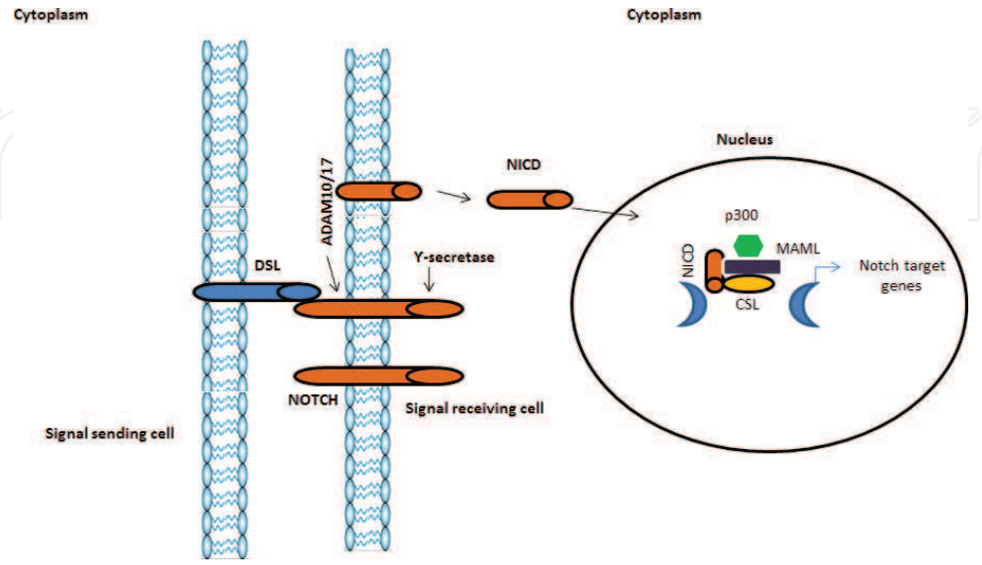

Figure 4. Basics of notch signaling pathway. Modified from Acar et al. [42].

treatment in HER2 positive breast xenografts and partially reverses trastuzumab resistance *in vivo*, MK-0752 reduced breast cancer stem cell subpopulation *in vitro* and in human tissues and PF-03084014 exhibited antitumor and antimetastatic activity in breast xenograft models (**Table 3**) [45].

5.2. The Wnt/β-catenin pathway

The Wnt/β-catenin pathway plays an important role in both normal development and tumorigenesis. The initiation of Wnt/β-catenin pathway occurs via conserved growth factors of the wingless and integration site growth factor (Wnt) family. Nineteen different Wnt genes which share a high level of sequence homology encode Wnts. Binding of Wnts to cell surface receptors lead to the activation of Wnt pathway by triggering signaling cascades that are very crucial in many cellular functions including survival, cell proliferation, specification of cell fate, migration and polarity and self-renewal property of stem cells [46]. Canonical Wnt pathway is also known as β-catenin-dependent Wnt pathway. In the absence of this signaling, β-catenin is maintained at a low level via *ubiquitin/proteasome-mediated degradation*. This can be regulated through protein destruction complex consisting of adenomatous polyposis coli (APC), axin and glycogen synthase kinase-3β (GSK-3β). Binding of Wnt ligand to a seven-pass transmembrane Frizzled (FZD) receptor in combination with its co-receptor, low-density lipoprotein receptor-related protein 6 (LRP6) or its close relative LRP5leads to the activation of Wnt signaling pathway.. The formation of Wnt/FZD/LRP6 complex with the recruitment of the scaffolding protein Disheveled (Dvl) leads to LRP6 phosphorylation and recruitment of the Axin complex to the receptors. As a result, Axin-mediated β-catenin phosphorylation gets inhibited and β-catenin will be stabilized in the cytoplasm. Accumulated β-catenin translocates to the nucleus to form complexes with T cell factor/lymphoid enhancer factor (TCF/LEF) family of proteins and activates target gene expression of Wnt signaling pathway (**Figure 5**) [47].

It has been noticed that aberrant activation of canonical Wnt pathway, which supports the formation and the maintenance of cancer stem cells (CSCs), maintains the pluripotency of the stem cells instead of allowing them to differentiate leading to neoplastic proliferation. Dysregulation in any components of this pathway accelerates tumor growth [46].

Triple-negative breast cancers (TNBC) is considered to be the most difficult subtype to treat due to the lack of effective targeted therapy. It has been observed that Wnt/β-catenin signaling is activated in TNBC patients with the upregulation of its components. The overactivation of this signaling with the upregulation of Wnt receptor expression in TNBC and basal-like breast cancer (BLBC) advocates this signaling pathway might be utilized as therapeutic target for TNBC/BLBC. For instance, salinomycin has been identified as an inhibitor of LRP6 expression and Wnt/β-catenin signaling and also as a selective killer of breast cancer stem cells. Another example is mesoderm development (Mesd), which has been identified as a specialized chaperone for LRP5/6 and acts as an LRP6 antagonist. Several other Wnt pathway inhibitors are in clinical trials such as OMP-18R5, OMP-54F28 and OTSA101 targeting FZD7, FZD8 and FZD10, respectively, as summarized in **Table 3**. Drugs targeting β-catenin

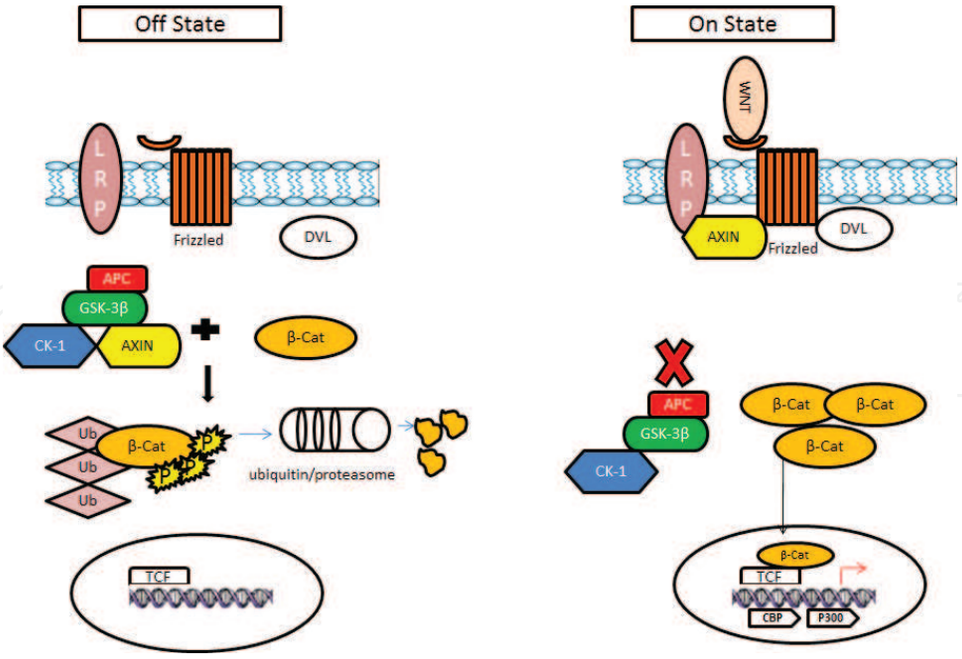

Figure 5. Schematic representation of canonical Wnt signaling pathway. Modified from Kazi et al. [46].

are also being tested [48]. Thus, effective treatment of TNBC by targeting Wnt signaling pathway is highly anticipated and would prove immensely beneficial in conquering this deadly disease.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviation

APC	adenomatous polyposis coli
ACS	American Cancer Society
AIs	aromatase inhibitors
GSK-3β	axin and glycogen synthase kinase-3β

BLBC	basal-kike breast cancer
CSCs	cancer stem cells
DSL	Delta/Serrate/LAG-2
Dll1	delta-like1
Dvl	Disheveled
DCIS	ductal carcinoma in situ
ER	endoplasmic reticulum
HER2	epidermal growth factor receptor 2
ER	estrogen receptor
FZD	frizzled
IHC	immunohistochemistry
IBC	inflammatory breast cancer
LRP6	lipoprotein receptor-related protein 6
MAML	member of the mastermind-like
ADAM10	metalloproteinase domain-containing protein 10
MAPK	mitogen-activated protein kinase
MAbs	monoclonal antibodies
NICD	notch intracellular domain
PI3K	phosphoinositide 3-kinase
PR	progesterone receptor
PKB Or AKT	protein kinase B
RBPj	recombination signal binding protein for immunoglobulin kappa J region
STATS	signal transducer and activator of transcription
siRNA	small interfering RNA
TKIs	tyrosine kinase inhibitors
TCF/LEF	T cell factor/lymphoid enhancer factor
RTKs	transmembrane receptor tyrosine kinases family
TNBC	triple-negative breast cancer
GSI	γ-secretase inhibitor

Author details

Ibrahim O. Alanazi[1]* and Zahid Khan[2]

*Address all correspondence to: ialenazi@kacst.edu.sa

1 National Center for Biotechnology, King Abdulaziz City for Science and Technology (KACST), Saudi Arabia

2 Genome Research Chair, Department of Biochemistry, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia

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