



REVIEW ARTICLE

# Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis



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**KEYWORDS**

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**Abstract** As the most commonly occurring cancer in women worldwide, breast cancer poses a formidable public health challenge on a global scale. Breast cancer consists of a group of biologically and molecularly heterogeneous diseases originated from the breast. While the risk factors associated with this cancer varies with respect to other cancers, genetic predisposition, most notably mutations in *BRCA1* or *BRCA2* gene, is an important causative factor for this malignancy. Breast cancers 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 breast cancer based on their invasiveness relative to the primary tumor sites. It is important to distinguish between the various subtypes because they have different prognoses and treatment implications. As there are remarkable parallels between normal development and breast cancer progression at the molecular level, it has been postulated that breast cancer may be derived from mammary cancer stem cells. Normal breast development and mammary stem cells are regulated by several signaling pathways, such as estrogen receptors (ERs), HER2, and Wnt/ $\beta$ -catenin signaling pathways, which control stem cell proliferation, cell death, cell differentiation, and cell motility. Furthermore, emerging evidence indicates that epigenetic regulations and noncoding RNAs may play important roles in breast cancer development and may contribute to the heterogeneity and metastatic aspects of breast cancer, especially for triple-negative breast cancer. This review provides a comprehensive survey of the molecular, cellular and genetic aspects of breast cancer.

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## Introduction

For many years, breast cancer has had the highest incidence of all cancers in women worldwide.<sup>1–4</sup> Patients have better survival compared with more fatal cancers possibly because the breast tissue is physically not a necessary organ for human survival. Yet the mental and emotional disturbances from major surgeries as well as deaths by relapse or metastasis seriously endanger women's health. Since the earliest known descriptions of breast cancer originating in ancient Egypt, people have been dedicated to finding means of eradicating this disease. Leaps and bounds have been made in terms of this endeavor, especially in recent years. Mastectomy and chemotherapy have greatly improved the survival of breast cancer patients and more elegant forms of surgical procedures are now being applied to minimize the post-treatment psychological impact.<sup>5–7</sup> However, without fully understanding the underlying mechanism and pathogenesis, the efficiency of prevention and treatment will always be limited.

Breast cancer is a compilation of distinct malignancies that manifests in the mammary glands. Carcinomas make up the majority of breast cancers while sarcomas such as phyllodes tumors and angiosarcomas are rarely seen. Thanks to the rapid progresses in molecular biology, systems biology and genome sciences in the past decades, our understanding about this disease has been dramatically expanded at cellular, molecular and genomic levels. Here, we intend to provide a comprehensive up-to-date overview of the basic biological aspects of breast cancer, including the risk factors, specific breast cancer classifications and subtypes, possible roles of mammary stem cells in breast cancer, major signaling pathways in breast cancer development, common gene mutations in breast cancer, the

regulatory roles of epigenetics and noncoding RNAs in breast cancer, the molecular basis of triple-negative breast cancer, tumor heterogeneity of breast cancer, and the mechanism underlying breast cancer metastasis. It is our goal to present the aforementioned information in hopes of disseminating the present understanding of the molecular and genetic bases of breast cancer, which can be employed to assist in the development of novel and targeted therapies as a means of realizing the full potential of personalized medicine for breast cancer.

## Disease landscape and risk factors of breast cancer

### U.S. breast cancer statistics

It is estimated that about one in eight US women will develop invasive breast cancer over the course of her lifetime. In 2018, over 266,000 new cases of invasive breast cancer are expected to be diagnosed in women in the U.S., along with nearly 64,000 new cases of non-invasive breast cancer in US women.<sup>1,8,9</sup> As of January 2018, there are already more than 3.1 million US women with a history of breast cancer, which includes the women currently being treated as well as women who have finished treatment. In addition to the statistics regarding women, approximately 2500 new cases of invasive breast cancer are expected to be diagnosed in US men in 2018. Even so, breast cancer incidence rates in the US have steadily declined since 2000.<sup>1</sup> On account of treatment advances, increased awareness, and the possibility for earlier detection through screening, breast cancer death rates have been decreasing since 1989. Even with this marked progress, over 40,000 US

women are expected to die in 2018 from breast cancer. In fact, for women in the US, breast cancer death rates are higher than those of any other cancer except lung cancer.<sup>1,8,9</sup>

In addition to having the second highest cancer-related death rate, breast cancer is among most commonly diagnosed cancer in US women. In total, about 30% of newly diagnosed cancers in women will be breast cancers. Among US women under the age of 45, breast cancer is more common in African-American women than white women. African-American women are more likely to die of breast cancer. On the other hand, the risk of developing and dying from breast cancer is lower for Asian, Hispanic, and Native-American women in the US<sup>1</sup>.

### Genetic predispositions as important risk factors of breast cancer

At its most basic, a risk factor is defined as anything that affects individual's chance of getting a disease, in this case breast cancer. Certain major risk factors for breast cancer are beyond individual's control.<sup>10–21</sup> For example, simply being a woman is the main risk factor for breast cancer as this disease is about 100 times more likely to occur in women than in men. Aging inevitably increases one's risk of breast cancer as evinced by the fact that most breast cancers are diagnosed in women age 55 and older. Beyond the inherent risks of gender and aging as they relate to breast cancer, it has been well documented that a woman's risk of developing breast cancer nearly doubles if she has a first-degree relative (mother, sister, or daughter) diagnosed with breast cancer. Close to 15% of US women who suffer from breast cancer also have a family member who has been diagnosed.<sup>8,9,22</sup>

Overall, about 5–10% of breast cancers are linked to gene mutations inherited from a parent. The most common cause of hereditary breast cancer is an inherited mutation in the *BRCA1* or *BRCA2* gene.<sup>4,8,9,22–24</sup> Statistically, women with a *BRCA1* mutation have a 55–65% lifetime risk of developing breast cancer. For women with a *BRCA2* mutation, the lifetime risk is 45%. On average, a woman with a *BRCA1* or *BRCA2* gene mutation has about 70% chance of getting breast cancer by age 80. The effect of the mutation is related to how many other family members have breast cancer, as breast cancer risk goes up if more family members are affected. In the US, *BRCA* mutations are more common in Jewish people of Ashkenazi (Eastern European) origin than in other racial and ethnic groups although anyone can have these mutations. Women with one of these two mutations are also more likely to be diagnosed with breast cancer at a younger age, as well as to have cancer in both breasts. The impact of the *BRCA1* and *BRCA2* mutation expands beyond just breast cancer as having mutations in either of these genes is associated with an increased ovarian cancer risk as well. Conversely, *BRCA1* mutations are found less frequently in breast cancers occurring in men while *BRCA2* mutations are associated with a lifetime breast cancer risk of only about 6.8%.<sup>4,8,9,22–24</sup>

Although less common and less drastic in their increase of breast cancer risk than the *BRCA* mutations, inherited mutations in many other genes can also lead to

breast cancer development.<sup>4,8,9,22–24</sup> Some of the mutated genes include *ATM* (inheriting 2 abnormal copies of this gene causes the disease ataxia-telangiectasia), *TP53* (inherited mutations of this gene cause Li-Fraumeni syndrome with an increased risk of breast cancer, as well as some other cancers such as leukemia, brain tumors, and sarcomas), *CHEK2* (a *CHEK2* mutation can increase breast cancer risk about 2-fold), *PTEN* (inherited mutations in this gene can cause Cowden syndrome which is accompanied by a higher risk for both non-cancerous and cancerous tumors in the breasts, as well as growths in the digestive tract, thyroid, uterus, and ovaries), *CDH1* (inherited mutations cause *hereditary diffuse gastric cancer* with an increased risk of invasive lobular breast cancer), *STK11* (mutations in this gene can lead to Peutz-Jeghers syndrome with a higher risk of many types of cancer, including breast cancer), and *PALB2* (*PALB2* gene makes a protein that interacts with the protein made by the *BRCA2* gene, resulting in mutations in this gene causing a higher risk of breast cancer).<sup>4,8,9,22–24</sup>

Properly and carefully consulted genetic testing of mutations in the *BRCA1* and *BRCA2* genes, as well as other less commonly mutated genes such as *PTEN* or *TP53* in women in the high risk group can be beneficial for early detection and/or prevention of breast cancer development.<sup>4,8,9,22–24</sup> However, it is important to understand the limitations of genetic testing and what it can and can't tell an individual. In terms of practically making use of genetic testing for detection and prevention of breast cancer, it's also necessary to keep in mind that the testing is quite expensive and may not be covered by all health insurance plans. While genetic testing can be helpful in some cases, not every woman needs to be tested.

### "Non-genetic" risk factors of breast cancer

**Family history of breast cancer:** While less than 15% of women with breast cancer have a family member with this disease, women who do have close blood relatives with breast cancer have a higher risk.<sup>10–22,25</sup> For instance, having a first-degree relative (mother, sister, or daughter) with breast cancer almost doubles a woman's risk while having two first-degree relatives with the disease increases the woman's risk about 3-fold. Interestingly, women with a father or brother who have breast cancer also have a higher risk of breast cancer. Within the context on an individual, a woman with cancer in one breast has a higher risk of developing a new cancer in the other breast or in another part of the same breast.<sup>4,8,9,22–24</sup>

**Race and ethnicity:** In general, Caucasian women are slightly more likely to develop breast cancer than African-American women although breast cancer is more common in African-American women under age 45. Furthermore, African-American women are more likely to die from breast cancer at any age. Other races such as Asian, Hispanic, and Native American women have a lower risk of developing and dying from breast cancer.<sup>10–21</sup>

**Certain benign breast conditions:** Women with dense breasts on mammogram have a risk of breast cancer that is about 1.5–2 times that of women with average breast

density even though multi factors play a role in determining breast density, such as age, menopausal status, the use of certain drugs (such as menopausal hormone therapy) and pregnancy. Certain non-proliferative lesions may marginally affect breast cancer risk. These non-proliferative lesions include fibrosis and/or simple cysts, mild hyperplasia, 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.<sup>26,27</sup>

**Certain proliferative breast lesions:** Some proliferative lesions without atypia seem to raise a woman's risk of breast cancer slightly.<sup>10–21</sup> Examples of such proliferative lesions are ductal hyperplasia, fibroadenoma, sclerosing adenosis, papillomatosis or radial scar. However, certain proliferative lesions with atypia in the ducts or lobules of the breast tissue will increase breast cancer risk 4–5-fold; and these include atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia (ALH).<sup>28,29</sup>

**Lobular carcinoma in situ (LCIS) or lobular neoplasia:** LCIS cells are cancer-like and grow in the lobules of the milk-producing glands of the breast, but are limited within the walls of the lobules.<sup>10–21</sup> LCIS is traditionally grouped with ductal carcinoma in situ (DCIS) as a non-invasive breast cancer, while recent updates in the field consider LCIS to be benign. However, LCIS differs from DCIS in that it usually does progress to become invasive cancer if it is not treated. Women with LCIS also have a much higher risk of developing cancer in either breast.

**Chest radiation therapy:** Women, who were treated with radiation therapy to the chest for another cancer when they were younger, have higher risk for developing breast cancer.<sup>10–21</sup> The impact of this factor on increasing risk is highest if the individual had radiation as a teen or young adult, when the breasts were still developing. Conversely, radiation treatment after age 40 does not seem to increase breast cancer risk.

**Exposure to diethylstilbestrol (DES):** From 1940s through early 1970s some pregnant women were given an estrogen-like drug DES because it was thought to lower the incidence of miscarriage.<sup>10–21</sup> These women have a slightly increased risk of breast cancer, and women whose mothers took DES during pregnancy may also have a slightly higher risk of breast cancer.

**Lifestyle and Personal Behavior-Related Risk Factors of Breast Cancer** Vast majority (about 85%) of breast cancers occur in women without apparent family history of breast cancer. These cancers may be caused by genetic mutations that occur as a result of the aging process and lifestyle-related risk factors, rather than inherited mutations.

**Birth control and contraceptives:** Many birth control methods use hormones, which may increase breast cancer risk.<sup>10–21</sup> Women using oral contraceptives have a slightly higher risk of breast cancer than women who have never used them, although the risk seems to go back to normal over time once the regimen is stopped. As an injectable form of progesterone, Depo-Provera has been shown to have an increase in breast cancer risk, but there is seemingly no increased risk in women five years after they have stopped receiving the shots. Birth control implants,

intrauterine devices (IUDs), skin patches, and vaginal rings usually also use hormones and thus in theory may increase breast cancer risk. Consequently, whenever considering the use of hormonal birth control, women should discuss the coupling of this impact with any other risk factors for breast cancer with their health care providers.

**Hormone replacement therapy (HRT) after menopause:** The hormone estrogen (often combined with progesterone) has been used to relieve symptoms of menopause and to prevent osteoporosis.<sup>10–21,30</sup> Combined hormone therapy is needed in most cases as use of estrogen alone can increase the risk of cancer of the uterus. However, for women who have had a hysterectomy, estrogen by itself can be used. Postmenopausal combined hormone therapy increases the risk of breast cancer, the chances of dying from breast cancer, and the likelihood that the cancer may be found only at a more advanced stage. This increase in risk is usually seen with as little as two years of use. However, the increased risk from combined HRT is reversible and its risk applies only to current and recent users, as a woman's breast cancer risk seemingly returns to that of the general population within five years of stopping HRT. The use of bioidentical or "natural" estrogen and/or progesterone is not necessarily safer or more effective, and thus should be considered to have the same health risks as any other type of HRT. Short term use of estrogen alone after menopause does not seem to increase the risk of breast cancer much. However, long-term use of estrogen therapy (e.g., >15 years) was reported to increase the risk of ovarian and breast cancer. Thus, the decision to use any forms of HRT should be made by a woman and her physician after weighing the possible risks and benefits, and considering her other risk factors for heart disease, breast cancer, and osteoporosis.

**Excessive alcohol consumption:** Drinking alcohol is clearly linked to an increased risk of breast cancer, and the increase in risk caused by this factor correlates with the amount of alcohol consumed.<sup>10–21,31</sup> For example, women who have two to three drinks a day have approximately 20% higher risk of breast cancer compared to women who don't drink alcohol. Women who have only one alcoholic drink per day have a very small increase in risk.

**Significant overweight or obese:** Before menopause women's ovaries make most of the body's estrogen, while fat tissue makes only a small amount.<sup>10–21</sup> However, when the ovaries stop making estrogen after menopause, most of a woman's estrogen comes from fat tissue. Thus, having more fat tissue after menopause will raise estrogen levels and increase breast cancer risk. Furthermore, being overweight tends to lead to higher blood insulin levels, and higher insulin levels are linked to certain cancers, including breast cancer. Nonetheless, the link between body weight and breast cancer risk is complex and remains to be fully understood.

**Not having children or not breastfeeding:** Women who have not had children or who have their first child after age 30 have a slightly higher overall risk for breast cancer. Conversely, having multiple pregnancies and/or becoming pregnant at an early age reduce breast cancer risk.<sup>10–21</sup> Nonetheless, pregnancy seems to have different effects on different types of breast cancer, and pregnancy seems to increase risk for triple-negative breast cancer. It has



been suggested that breastfeeding may slightly lower breast cancer risk, especially if it is continued for 1.5–2 years. A possible explanation for this effect is that breastfeeding reduces woman's total number of lifetime menstrual cycles.

**Starting menstruation early or stopping menopause after age 55:** Women will have more menstrual cycles if they start menstruating early, especially before age 12, and thus they will have a longer lifetime exposure to the hormones estrogen and progesterone, leading to a slightly higher risk of breast cancer.<sup>10–21</sup> Similarly, women will have more menstrual cycles if they go through menopause later, especially after age 55, and also have a longer lifetime exposure to estrogen and progesterone with a higher risk of breast cancer.

**Lack of physical activity:** Growing evidence indicates that regular physical activity, especially in women past menopause, may reduce breast cancer risk.<sup>10–21</sup> It is not completely clear how physical activity might reduce breast cancer risk, but it may be due to the fact that activity levels affect body weight, inflammation, hormones, and energy balance.

## Classifications and stages of breast cancer

### Types of breast cancer based on pathology, invasiveness and prevalence

There are many types of breast cancers as it can present in distinct areas of the breast, such as the ducts, the lobules, or the tissue in between. The type of breast cancer is determined by the specific cells that are affected. Based on which cell origin is involved, breast cancers can be divided into two broad classifications, carcinomas and sarcomas. Carcinomas are breast cancers arising from the epithelial component of the breast, which consists of the cells that line the lobules and terminal ducts responsible for making milk. Sarcomas are a much rarer form of breast cancer (<1% of primary breast cancer) arising from the stromal components of the breast, which include myofibroblasts and blood vessel cells. These groups are not always sufficient categories as, in some cases, a single breast tumor can be a combination of different cell types.<sup>4,8,9,22–24</sup>

Most breast cancers are carcinomas. Within the large group of carcinomas, there are many different types of breast cancer identified based on their invasiveness relative to the primary tumor sites. Accurately being able to distinguish between the various subtypes is vital as they each have different prognoses and treatment implications. Based on criteria of pathological features and invasiveness, common breast cancers can be divided into three major groups: non-invasive (or in situ), invasive, and metastatic breast cancers.<sup>4,8,9,22–24</sup>

### Non-invasive (or in situ) breast cancer

**Ductal carcinoma in situ (DCIS;** also called intraductal carcinoma): As one of the most common types of breast cancer, DCIS is a non-invasive or pre-invasive breast cancer, which develops inside of pre-existing normal ducts.<sup>4,8,9,22–24</sup> While DCIS is itself not invasive, in situ

carcinomas have high potential to become invasive cancers, so early and adequate treatment is important in preventing the patient from developing an invasive cancer.

**Invasive or infiltrating breast cancer** Invasive breast cancers have cancer cells that invade and spread outside of the normal breast lobules and ducts, growing into the surrounding breast stromal tissue.<sup>4,8,9,22–24</sup> About two-thirds of women with an invasive form of breast cancer are 55 or older when they are diagnosed. Invasive carcinomas have the potential to spread to other sites of the body, such as the lymph nodes or other organs and to form metastases thus entering the classification of metastatic breast cancers.<sup>4,8,9,22–24</sup> Based on the tissue and cell types involved, invasive breast cancers are further divided into following two types:

**Invasive Ductal Carcinoma (IDC):** IDC is the most common type of breast cancer with about 80% of all breast cancers being constituted by invasive ductal carcinomas.<sup>4,8,9,22–24</sup> The IDC classification includes several subtypes: 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.<sup>4,8,9,22–24</sup>

**Invasive Lobular Carcinoma (ILC):** ILC is the second most common type of breast cancers and accounts for approximately 10–15% of all breast cancers.<sup>4,8,9,22–24</sup> Although ILC can affect women at any age, it is more common in older women. ILC tends to occur later in life than IDC, e.g. in the early 60s as opposed to the mid-to late 50s for IDC.<sup>4,8,9,22–24</sup>

Together, 90–95% of all breast cancer cases fall into invasive subcategories. IDC and ILC cancers each exhibit distinct pathologic features. Lobular carcinomas grow as single cells arranged individually, in single file, or in sheets, and they have distinct molecular and genetic aberrations that distinguish them from ductal carcinomas. Ductal and lobular carcinomas may have different prognoses and treatment options and are thus important to clearly differentiate from one another.

### Metastatic breast cancer

Metastatic breast cancers, also known as stage IV or advanced breast cancers, are late stage breast cancers, which have spread to other organs in the body.<sup>4,8,9,22–24</sup> Metastases from breast cancers can be found in lymph nodes in the armpit, and/or in distant sites such as the lung, liver, bone and brain. Even after the primary tumor is removed, microscopic tumor cells or micro-metastases may remain in the body, which allows the cancer to return and disseminate. Clinically, patients may initially be diagnosed with metastatic disease (or de novo metastatic breast cancers), or they may develop metastases months or years after receiving initial treatment. The risk of breast cancer returning and metastasizing is not clearly understood or predictable as it varies from person to person, largely depending on the unique molecular biology of the tumor and the stage at the time of the original diagnosis. Unfortunately, approximately 30% of the women diagnosed with early-stage breast cancer will develop a metastatic form of the disease.<sup>4,8,9,22–24</sup>

## Less common types of breast cancer

While IDC and ILC account for approximately 90–95% of all breast cancer cases, several rare types of breast cancers can be observed in a clinical setting.<sup>4,8,9,22–24</sup>

**Inflammatory breast cancers (IBC):** IBC is an uncommon type of invasive breast cancer that comprises 1%–5% of all breast cancers.<sup>4,8,9,22–24</sup> IBC differs from other types of breast cancers in its symptoms, prognosis and treatment. Typical IBC symptoms include inflammation-like breast swelling, purple or red color of the skin, and pitting or thickening of the skin of the breast, all of which are likely caused by cancer cells blocking lymph vessels in the skin. IBC often does not present with a breast lump and may not be identifiable on mammograms. IBC tends to occur in younger women, and is more common in African-American women as well as in women who are overweight or obese. Furthermore, IBC tends to be more aggressive, growing and spreading much more quickly than the common types of breast cancers. IBC is always first diagnosed at a locally advanced stage where the breast cancer cells have grown into the skin. In about one-third of cases, IBCs are already metastasized to distant sites of the body at diagnosis, which makes it more difficult to treat successfully.<sup>4,8,9,22–24</sup>

**Breast cancers in men and children & adolescents:** Male breast carcinomas, which can be either *in situ* or invasive, account for <1% of all breast cancer cases.<sup>4,8,9,22–24</sup> Male breast cancers can look identical to those seen in women as most cases consist of invasive ductal carcinomas with estrogen receptor (ER) expression. The most common breast lesion in males is gynecomastia (or breast enlargement), which may involve either the unilateral breast or bilateral breasts. Breast lesions are also uncommon in children and adolescents, but do occur. These rare cases can include both benign lesions, such as juvenile fibroadenoma, and malignant lesions, such as secretory carcinoma. Pediatric patients are more likely to suffer from metastatic tumors to the breast from lymphoma or alveolar rhabdomyosarcoma.

**Paget disease of the breast:** This rare form of breast cancer starts in the breast ducts, spreads to the skin of the nipple and then expands to the areola (the dark circle around the nipple).<sup>4,8,9,22–24</sup> Paget disease makes up <3% of all breast cancer cases. Paget's cells look significantly different from normal cells and divide rapidly. About half of the cells are positive for estrogen and progesterone receptors, and most cells are positive for the HER2 protein. The cancer is typically diagnosed with a biopsy of the tissue, sometimes followed by a mammogram, sonogram or MRI to confirm the diagnosis. It should also be pointed out that Paget's disease of the breast is not related in any medical way to other conditions named after Sir James Paget, such as Paget's disease of the bone.

**Papillary carcinoma:** Papillary carcinoma is another rare form of breast cancer that accounts for <3% of all breast cancer cases.<sup>4,8,9,22–24</sup> Papillary carcinoma cancer cells are usually arranged in finger-like projections. In some cases, the cancer cells are quite small in size and form micropapillary. Most papillary carcinomas are invasive, and are treated in the same manner as IDCs. However, invasive

papillary carcinoma usually has a better prognosis than other invasive breast cancer. It is noteworthy that papillary carcinomas may also be detected when they are still noninvasive. A noninvasive papillary carcinoma is usually considered a variety of DCIS.

**Phyllodes tumor:** Phyllodes tumors are rare breast tumors that develop in the stromal cells of the breast.<sup>4,8,9,22–24</sup> Most of these tumors are benign, but about one-quarter are malignant. Phyllodes tumors are most commonly found in women in their 40s, and in women with Li-Fraumeni syndrome who have an increased risk for this type of tumor.

**Angiosarcoma of the breast:** As a form of sarcoma, angiosarcoma is a rare cancer that originates the epithelial cells that line blood or lymph vessels.<sup>4,8,9,22–24</sup> Angiosarcomas make up <1% of all breast cancers and it can involve the breast tissue or the skin of the breast. Some arise from prior radiation therapy in that area. Although rare, angiosarcomas tend to grow and spread quickly and need to be treated accordingly.

## Molecular or intrinsic subtypes of breast cancer

Breast cancer encompasses a heterogeneous and phenotypically diverse group of diseases. It is composed of several biological subtypes that have distinct behaviors and responses to therapy.<sup>4,8,9,22–24</sup> Gene expression studies have identified several distinct breast cancer subtypes that differ significantly in prognosis as well as in the therapeutic targets present in the cancer cells. With the advance of gene expression profiling techniques, the list of intrinsic genes that differentiate these subtypes is now made up of several clusters of genes relating to estrogen receptor (ER) expression (the luminal cluster), human epidermal growth factor 2 (HER2) expression, proliferation, and a unique cluster of genes called the basal cluster.<sup>4,8,9,22–24</sup> Through a utilization of these understandings, breast cancers are usually divided into five intrinsic or molecular subtypes that are based on the expression pattern of certain genes (Table 1).

**Luminal A breast cancer:** This subtype is estrogen-receptor (ER) and/or progesterone-receptor (PR) positive, HER2 negative, and has low levels of Ki-67. Luminal A cancers account for about 40% of all breast cancers. They are low-grade, slow growing, and tend to have the best prognosis. Treatment typically involves hormonal therapy.<sup>4,8,9,22–24</sup>

**Luminal B breast cancer:** Accounting for <20% of all breast cancers, this subtype is ER and/or PR positive, either HER2 positive or HER2 negative, and presents high levels of Ki-67. Luminal B cancers grow slightly faster than luminal A cancers, and their prognosis is slightly worse.<sup>4,8,9,22–24</sup>

**HER2-enriched breast cancer:** This subtype makes up 10%–15% of breast cancers and is characterized by the absence of ER and PR expression, the high expression of the HER2 and proliferation gene clusters, and the low expression of the luminal and basal clusters.<sup>4,8,9,22–24</sup> HER2-enriched cancers grow faster than luminal cancers and have a generally worse prognosis. However, they can be successfully treated with targeted therapies aimed at the HER2 protein, such as Herceptin (or trastuzumab), Perjeta (or pertuzumab), Tykerb (or lapatinib), and Kadcyla (or T-

**Table 1** Molecular/intrinsic subtypes of breast cancers.

Subtypes	Molecular Signatures	Characteristics	Treatment options <sup>a</sup>
Luminal A	ER+, PR±, HER2-, Low Ki67	~70%, Most common Best prognosis	Hormonal Therapy Targeted Therapy
Luminal B	ER+, PR±, HER2±, High Ki67	10%–20% Lower survival than Luminal A	Hormonal Therapy Targeted Therapy
HER2	ER-, PR-, HER2+	5%–15%	Targeted Therapy
Triple Negative	ER-, PR-, HER2-	15%–20% More common in black women Diagnosed at younger age Worst prognosis	Limited Targeted Therapy
Normal-like	ER+, PR±, HER2-, Low Ki67	Rare Low proliferation gene cluster expression	Hormonal Therapy Targeted Therapy

<sup>a</sup> Besides conventional surgical and non-surgical treatment.

DM1 or ado-trastuzumab emtansine). It must be clarified that the HER2-enriched subtype is not synonymous with clinically HER2-positive breast cancer. While about 50% of clinical HER2-positive breast cancers are HER2-enriched, the remaining 50% can include any molecular subtype but are mostly HER2-positive luminal subtypes. However, about 30% of HER2-enriched tumors are clinically HER2-negative.<sup>4,8,9,22–24</sup>

**Triple-negative/basal-like breast cancer (TNBC):** Accounting for approximately 20% of all breast cancers, the TNBC subtype is characterized as ER-negative, PR-negative and HER2-negative.<sup>4,8,9,22–24</sup> TNBC is more common in women with *BRCA1* gene mutations as well as among women younger than 40 years of age and African-American women. TNBC usually behaves more aggressively than other types of breast cancer making it a high grade breast cancer. The most common histology seen in TNBC is infiltrating ductal carcinoma, although a rare histologic subtype, medullary carcinoma, is generally also triple negative. Unlike other breast cancer subtypes with an arsenal of targeted regimens such as ER antagonists and HER2 monoclonal antibodies, TNBC's non-surgical treatment has been limited to conventional chemotherapy, until the recent approval of the PARP inhibitor Olaparib for *BRCA1* and *BRCA2* mutation carriers, who are more likely to develop TNBC.<sup>4,8,9,22–24</sup>

**Normal-like breast cancer:** This subtype is similar to luminal A disease. It is ER and/or PR-positive, HER2 negative, and has low levels of the protein Ki-67.<sup>4,8,9,22–24</sup> While normal-like breast cancer has a good prognosis, its prognosis is still slightly worse than luminal A cancer's prognosis.

The cellular and molecular heterogeneity of breast cancers mandates the analyses of multiple genetic alterations in concert, which has been made possible by the emergence of next-generation genomics and transcriptomics techniques.<sup>4,8,9,22–24</sup> Gene expression studies have thus identified several distinct breast cancer subtypes that differ markedly in prognosis and in their expression of therapeutic targets.<sup>32–38</sup> In fact, the intrinsic subtypes bifurcate into two segregated groups that correspond to the expression of hormone receptor-related genes. This segregation is consistent with both literature and clinical experience which show ER-positive and ER-negative cancers to define biologically distinct phenotypes that may derive from different progenitor cells.<sup>35</sup> Furthermore, the genomewide analyses and transcriptomic profiling have provided critical insights into the nuances of the molecular classification of breast cancers,<sup>34,38</sup> and have helped established several diagnostic and prognostic panels, such as the Oncotype Dx 21-gene Recurrence Score (RS),<sup>39</sup> the Breast Cancer Index (BCI),<sup>40</sup> the Predictor Analysis of Microarray 50 (PAM50) Risk of Recurrence (ROR) score,<sup>41–45</sup> the Amsterdam 70-gene prognostic profile (Mammprint),<sup>46–50</sup> and the Genomic Grade Index (GGI).<sup>51</sup>

### Clinical staging and survival rates of breast cancer

Once breast cancer is diagnosed, tests are performed to determine the stage of the disease, which will impact the treatment patients receive. The clinical staging of breast cancer is identical across breast cancer subtypes according to the American Joint Committee on Cancer (AJCC) and the International Union for Cancer Control (UICC) Tumor, Node, and Metastasis (TNM) breast cancer staging system: Stage 0, Stage I, Stage II, Stage III and Stage IV, as detailed in [Table 2](#).

### Cell origins of breast cancer

#### Mammary gland development and mammary stem cells

Human mammary gland development is a progressive process that is initiated during embryonic life.<sup>52,53</sup> The mammary gland consists of a highly branched network of epithelial tubes, embedded within a complex stroma ([Fig. 1](#)).<sup>53</sup> The mammary epithelium originates during embryonic development from an epidermal placode.<sup>53</sup> Regulated by epithelial–mesenchymal interactions, the placodes descend into the underlying mesenchyme and produce the rudimentary ductal structure of the gland present at birth.<sup>52,53</sup> At birth, the breast rudiment is formed by 10–12 primitive ductal elements located beneath the nipple–areola complex.<sup>52</sup> The breast undergoes dramatic changes in size, shape, and function in association with puberty, pregnancy, and lactation, in response to steroid hormone and growth factor receptor signaling.<sup>53</sup> One unique feature of the breast is that mammary epithelium is highly responsive to local and systemic signals and displays significant morphologic changes of the ductal tree during puberty and pregnancy.<sup>54</sup>

Transplantation and lineage tracing studies indicated that a hierarchy of stem and progenitor cells exists among

**Table 2** Anatomic stage groups of breast cancer.

Stages	Definition
Stage 0	Ductal Carcinoma In Situ
Stage I	IA Primary invasive tumor with a size of $\leq 20$ mm No nodal involvement
	IB Nodal micrometastases ( $>0.2$ mm, $<2.0$ mm) with or without $\leq 20$ mm primary tumor
Stage II	IIA Movable ipsilateral Level I, II lymph node metastases with $\leq 20$ mm primary tumor; Or $> 20$ mm, $\leq 50$ mm tumor with no nodal involvement
	IIB Movable ipsilateral Level I, II lymph node metastases with $>20$ mm, $\leq 50$ mm tumor; Or $> 50$ mm tumor with no nodal involvement
Stage III	IIIA Movable ipsilateral Level I, II lymph node metastases with $>50$ mm tumor; Or any size primary tumor with fixed ipsilateral Level I, II or internal lymph node metastases
	IIIB Primary tumor with chest wall and/or skin invasion
	IIIC Any size primary tumor with supraclavicular or ipsilateral Level III lymph node metastases; Or with ipsilateral Level I, II and internal lymph node metastases
Stage IV	Any case with distant organ metastasis

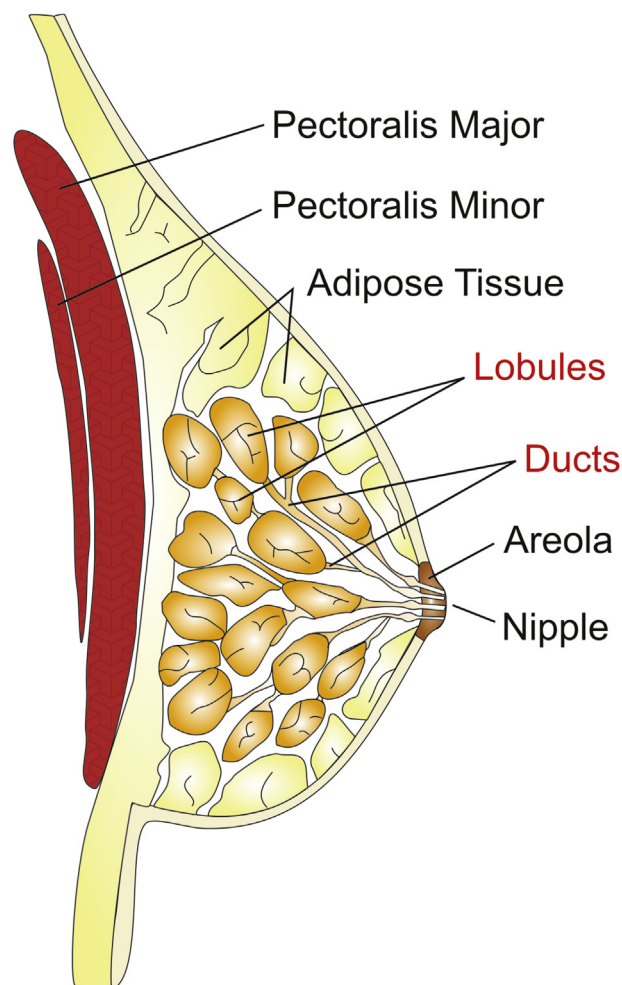
Notes: 1). Lobular carcinoma in situ is now considered benign thus removed from the breast cancer staging system.

2). The Anatomic Stage Group is to be used when biomarker tests are not available.

Source: AJCC Cancer Staging Manual, Eighth Edition, The American College of Surgeons (ACS), Chicago, IL, USA. With reprint permission of ACS.

the mammary epithelium.<sup>54,55</sup> In fact, lineage tracing has highlighted the existence of bipotent mammary stem cells (MaSCs) in situ as well as long-lived unipotent cells that drive morphogenesis and homeostasis of the ductal tree.<sup>54,56</sup> Accumulating evidence suggests the existence of a heterogeneous MaSC compartment comprising fetal MaSCs, slow-cycling cells, and both long-term and short-term repopulating cells.<sup>54–56</sup> Furthermore, diverse luminal progenitor subtypes have been identified in both normal mouse and normal human mammary tissue.<sup>56</sup> This suggests that the elucidation of the normal cellular hierarchy is an important step toward understanding the “cells of origin” for breast cancer (Fig. 2).<sup>54–57</sup>

Mammary stem cells (MaSCs) exist as a very small proportion of cells in the mammary gland that are undifferentiated and can produce new MaSCs through self-renewal as well as to give rise to a variety of differentiated cells through symmetric and asymmetric divisions.<sup>57</sup> Asymmetric divisions are hypothesized to give rise to progenitor cells.<sup>57–60</sup> In a healthy body, SCs are well understood in their longevity and self-renewal properties for their role in maintenance, repair, and tissue regeneration.<sup>60</sup> It is accepted that MaSCs possess functions under normal circumstances involved in responding to cellular needs during the course of reproductive life. The function of MaSCs is thought to occur through close interaction with their specific



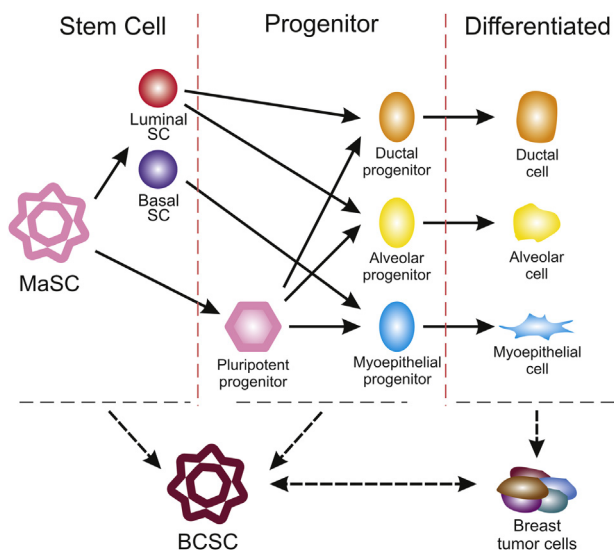
**Figure 1** Anatomical and histologic origins of breast cancer. Most breast cancers arise from the lobules or the ducts of the breast. In some cases, the tumor infiltrates the skin or components of the chest wall such as the pectoralis muscles. The tumor cells also are capable of converting the microenvironment into a tumor-friendly state to promote their growth and expansion.

cellular microenvironment which is also referred to as the mammary stem cell niche.<sup>57,60,61</sup> The specific locations of these cells, with their unique self-renewal and differentiation characteristics, remain a topic for debate as does their role in breast cancer. MaSCs are broadly referred to as slow-cycling and long-lived stem cells, or as progenitor cells, which exist as a singular, role-committed population. There is still much debate over the cells’ differentiation capabilities into one of both types of mammary epithelial cell lineages, as well as over the impact of cell plasticity.<sup>58</sup>

### Roles of mammary stem cells in breast cancer development

One attempt to explain MaSCs in relationship to breast cancer, as these cells do have a normal role in the body, is the umbrella concept or cancer stem cell (CSC) theory.<sup>62–65</sup> While CSC theory has a fairly extensive history,<sup>65</sup> the focus





**Figure 2 Mammary cell hierarchy and breast cancer stem cells.** Mammary stem cells (MaSCs) are multipotent self-renewing cells of great importance in the development and replenishment of mammary glands, as well as having implications in cellular origin of breast cancer stem cells (BCSCs), which has been extensively traced using various methods using human and mice samples. Two models have been established to define the relationship between MaSCs and breast cancer.

here will be on the most recent advancements and understandings of the theory as they relate to breast cancer (Fig. 2).<sup>54–57</sup> The primary bifurcation in this theory is whether CSCs are the cell of origin from which cancer cells develop, a hypothesis based on observations of the similarities between tissue replenishment and tumorigenesis, or if malignant cells attain stem cell potential.<sup>60,66</sup> Effectively, the debate centralizes around the importance of bipotent and unipotent luminal and myoepithelial stem cells during both normal cell development and carcinogenesis.<sup>54–57</sup>

One challenge in accumulating data to either support or negate this theory is the inaccuracy and variability between human and mouse models of selected biomarkers some of which include CD49f, EpCAM, CD10 and ALDH1A1, SSEA4, CD44, CK12<sup>+</sup>/CK19<sup>+</sup>.<sup>66</sup> Gaining an understanding of cellular heterogeneity, which CSC theory attempts to explain, is imperative to the development of more specialized treatments for breast cancer, in which cell heterogeneity poses a major hurdle. CSC theory rests on a widely accepted structure of cellular hierarchy in both normal and carcinogenic cells with stem cells residing at the apex and cells differentiating from that point out.<sup>66</sup> The consequence of this structure is that MaSCs, as distinct from mammary repopulating cells, are bi- or multipotent, a conclusion that has been well supported. These mammary repopulating cells can be identified as adopting multipotent characteristics under regenerative conditions viewed through transplantation.<sup>67,68</sup>

MaSCs are posited to exist in distinct microenvironment known as a stem cell niche, which places added importance on mutual feedback relationships between the epithelial cells that come from MaSCs, surrounding stromal cells, and

the extracellular matrix.<sup>57</sup> However, the signaling pathways and the ways in which they contribute to the normal and carcinogenic roles of MaSCs are not fully understood.<sup>54–57</sup>

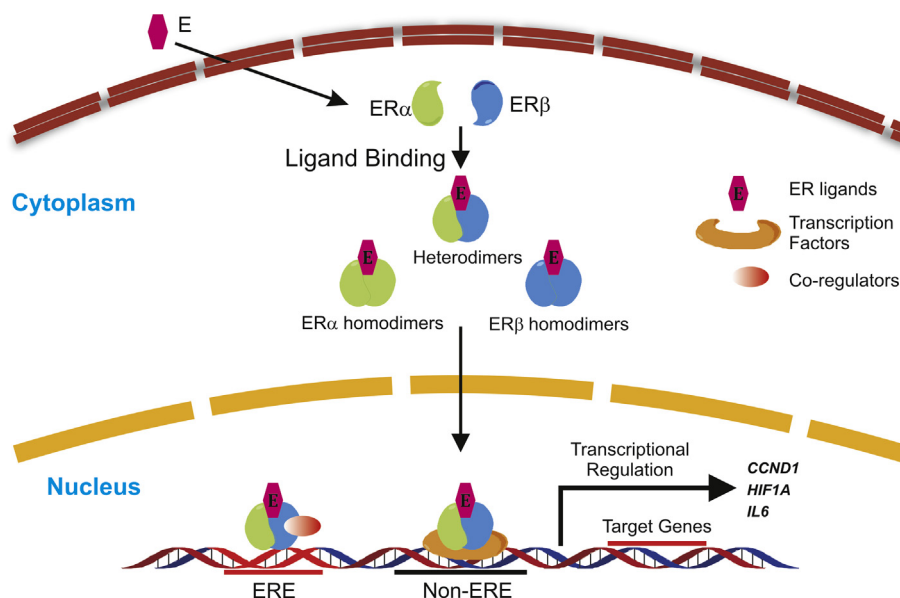
Two major cell lineages lie at the root of this argument in terms of breast CSC origin: basal cells and luminal cells.<sup>54–57</sup> Basal cells are one of the two main cell lineages in the in the mammary gland, and the other being luminal cells. Basal cells surround the luminal cell layer in the structure of the mammary gland. Luminal cells line the lumen of the mammary ducts and alveoli. Basal cells normally express cytokeratin-5, -14, and smooth muscle actin while luminal cells express cytokeratin-8 and can be either positive or negative for hormone receptors.<sup>54–57</sup> Recent studies have been able to exceed prior limitations to mouse models for cellular lineage-tracing to using patient-derived breast cancer organoids.<sup>69</sup> The construction of a living breast cancer organoid biobank is one such project that may be employed for research on cancer cell heterogeneity and drug discovery as a more accurate model.<sup>69–71</sup>

## Major signaling pathways in breast cancer development and progression

There are remarkable parallels between normal development and cancer progression at the molecular level.<sup>52,53</sup> Normal human development is tightly controlled by complex signaling pathways, which allow cells to communicate with each other and their surrounding environment.<sup>72,73</sup> Not surprisingly, many of these same signaling pathways are dysregulated or hijacked by cancer cells and CSCs.<sup>74</sup> In essence, cancer is driven by genetic and epigenetic alterations that allow cells to escape the mechanisms that normally control their proliferation, survival and migration.<sup>74</sup> Many of these alterations map to signaling pathways that govern cell proliferation and division, cell death, cell differentiation and fate, and cell motility.<sup>74</sup> Thus, activating mutations of proto-oncogenes can cause hyperactivation of these signaling pathways, whereas inactivation of tumor suppressors eliminates critical negative regulators of signaling.<sup>74</sup> Here, we will focus on the predominant signaling pathways that regulate normal mammary gland development and breast cancer stem cell functions, namely estrogen receptor (ER) signaling, HER2 signaling and canonical Wnt signaling.

## ER signaling and ER-Positive breast cancer

Estrogen receptors (ERs) consist of membrane estrogen receptors (mostly G protein-coupled receptors) and nuclear estrogen receptors (ER $\alpha$ , ER $\beta$ ).<sup>75–78</sup> Both ER $\alpha$  and ER $\beta$  are transcriptional factors that either activate or repress the expression of target genes upon ligand binding. ER $\alpha$  (coded by *ESR1*) and ER $\beta$  (coded by *ESR2*) share common structural features that serve their main functions while upholding receptor-specific signal transduction through exclusive elements.<sup>77–79</sup> ER $\alpha$  and ER $\beta$  both contain six functional domains with various degrees of similarity and retain the ability of forming heterodimers.<sup>75,77</sup> The region with the highest identity of 96% is the DNA-binding domain (DBD), which mediates the interaction of ER dimers and estrogen response elements (EREs) of target genes (Fig. 3).<sup>77–80</sup>



**Figure 3 ER signaling pathway.** Breast cancer cells have relatively high ER $\alpha$  expression and low ER $\beta$  expression. These two types of nuclear hormone receptors form homo- or heterodimers upon ligand binding and translocate into the cell nucleus for transcriptional regulation, which is the main function of ERs. ER dimers bind to the ERE region of target genes and recruit co-regulators to achieve the regulation of transcriptional activity. Another mechanism by which ERs control the expression of target genes is acting as a co-regulator for other transcription factors.

Apart from the ERE-dependent pathway, ERs also have the capability of transcription regulation without the involvement of EREs.<sup>77–79</sup> In ER-mediated transcription regulatory process, many co-activators and co-repressors,<sup>81,82</sup> including BRCA1<sup>83</sup> play important roles. BRCA1 acts as a tumor suppressor partially by inhibiting ER $\alpha$  signaling.<sup>84</sup> The intricacy of ER-mediated signaling suggests its complex functions in many biological and pathological settings. ER $\alpha$  plays a major role in the pathogenesis of breast cancers as approximately 75% of breast cancers have positive expression of this specific type of hormonal receptor.<sup>85</sup> The function of ER $\alpha$  in breast cancer biology is finely tuned by many factors and plays an active role in numerous occurrences of cross-talk.<sup>77,80</sup>

One of the best characterized mechanisms by which ER $\alpha$  promotes the growth of breast tumor cells is through its interaction with cyclin D1.<sup>86–88</sup> Cyclin D1 is an important activator of cyclin-dependent kinases (CDKs) 4 and 6, which coordinates the transition of cell cycle from G1 to S phase in many cancer cells.<sup>89</sup> The synergism within the ER $\alpha$  and cyclin D1 feedback loop could moderately explain the mechanism of antiestrogen therapy resistance and provide rationale for the combined use of selective CDK4/6 inhibitors and hormonal therapy agents in ER+ patients.<sup>90–92</sup>

Breast cancer biology and therapeutics does not only submit to the influence of the full-size ER $\alpha$ , but also many of its isoforms.<sup>93</sup> Depending on the structural domains, these isoforms exhibit paradoxical effects in the regulation of ER $\alpha$  signaling.<sup>94–96</sup> High expression of a truncated isoform of ER $\alpha$ , ER $\alpha$ 36, was found to be correlated with metastatic phenotype and poor prognosis in breast cancer patients.<sup>97</sup> The most commonly used ER $\alpha$  antagonist Tamoxifen had an adverse effect on ER $\alpha$ 36, exacerbating stem cell qualities by upregulating the transcription of ALDH1A1, thus promoting the progression of the disease

instead of suppressing it. This discovery could account for part of the reality that only about 70% of ER+ patients respond to hormonal therapy and potentially increase the precision of personalized treatment.<sup>97</sup>

While ER $\alpha$  is the one of the mostly frequently examined parameters in breast cancer patients, ER $\beta$  has been gradually drawing attention. ER $\beta$  has partial overlap with ER $\alpha$  in the tissue expression profile. Normal breast tissue frequently expresses ER $\beta$  and the level decline along with the progression of breast tumors.<sup>98</sup> Consistent with the pattern in expression, many studies provided both *in vitro* and *in vivo* evidence supporting its role as a breast cancer suppressor.<sup>99,100</sup> In a conditional *p53* knockout mouse model, the formation of mammary tumors was sped up by the simultaneous knockout of *Esr2* (mouse gene coding ER $\beta$ ).<sup>101</sup> Tumors in the double knockout mice also displayed more malignant phenotypes, suggesting ER $\beta$  execute its tumor-suppressing role at least partly by interacting with *p53*. ER $\beta$  signaling in breast cancer and other circumstances are not as well elucidated as ER $\alpha$  at the moment.<sup>101</sup> However, since more studies are implying a significant position for ER $\beta$  in the mammary tumorigenesis, it will not be surprising if ER $\beta$  turn out to be just as crucial as ER $\alpha$ , if not more.<sup>80</sup>

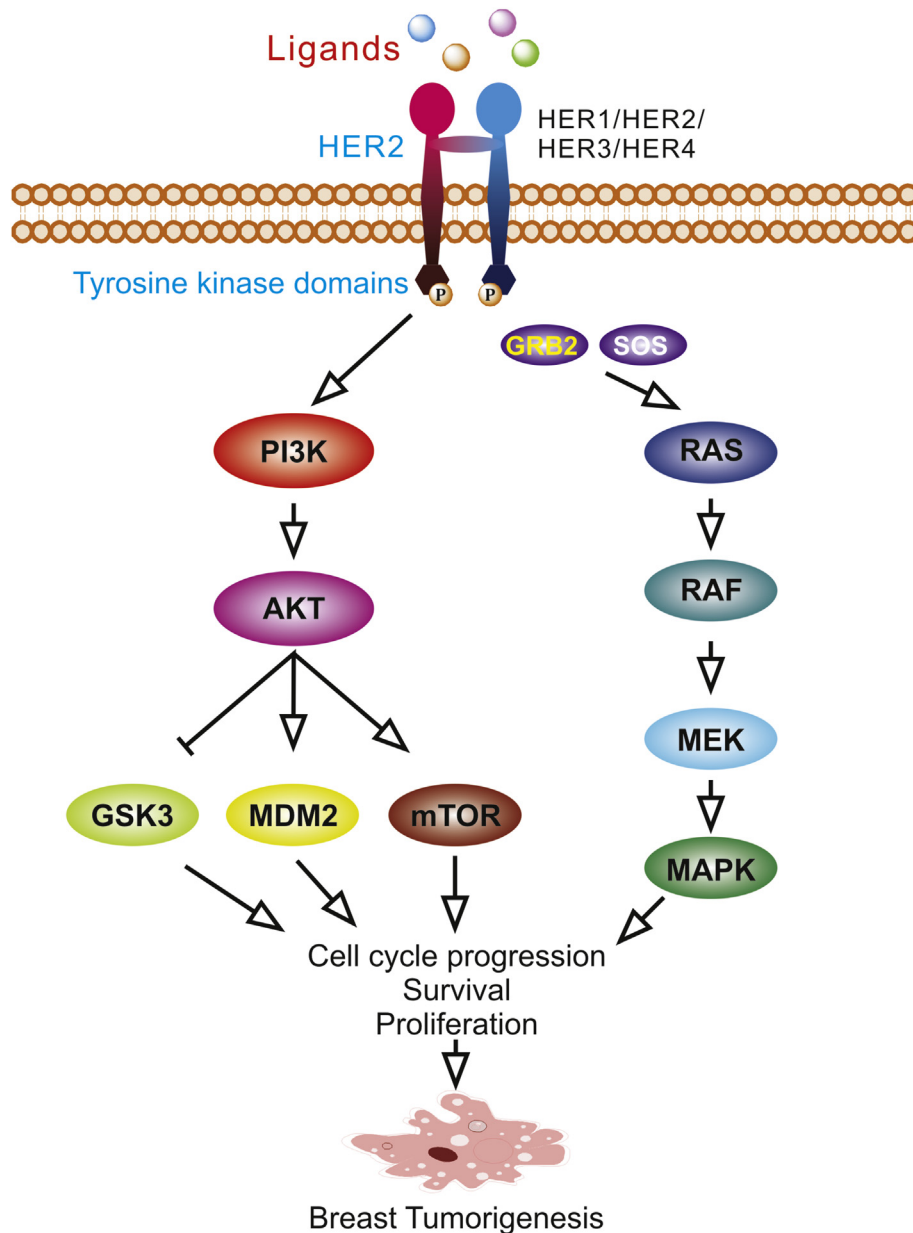
## HER2 signaling and HER2-Positive breast cancer

Human epidermal growth factor receptors (EGFRs, or HERs) 1 to 4 constitute a family of tyrosine kinase receptors expressed in normal tissues and in many types of cancer.<sup>102–106</sup> Human epidermal growth factor receptor-2 (or HER2/NEU, c-ERBB2) is a member of the EGFRs.<sup>104,106</sup> Like the others, HER2 is a receptor tyrosine kinase that consists of an extracellular ligand-binding domain, a trans-membrane domain, and an intracellular domain.<sup>105,107</sup> The

constitutively active form makes HER2 the preferred component to form dimers with other molecules and grants HER2 the capability of affecting many cellular functions through various pathways.<sup>108,109</sup> Ligand binding and subsequent dimerization stimulate phosphorylation of tyrosine residues in the intracellular domain of HER2, leading to the activation of multiple downstream signaling pathways such as the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K) pathways.<sup>110–112</sup> These signaling pathways are heavily associated with breast tumorigenesis (Fig. 4).<sup>105–107,113</sup>

HER2 is amplified in various human breast cancer cell lines.<sup>114</sup> HER2 signaling amplification results in HER2 protein overexpression which is linked to tumor cell

proliferation and cancer progression.<sup>115</sup> Targeted therapies are developed to bind specific molecules in signaling pathways important for cancer development and progression, providing most effective therapy in appropriately selected patients.<sup>105</sup> Novel mechanisms underlying the relationship between HER2 and breast cancer have been uncovered recently.<sup>105,116</sup> While the interplay of HER2 and ERs has long been recognized,<sup>102</sup> it was recently discovered that a new intermediary factor MED1 has significant impact on HER2-driven tumorigenesis.<sup>116</sup> The precancerous effect of HER2 was also found to be linked to inflammation and the expansion of cancer stem-like cells (CSCs) in breast cancer.<sup>117</sup> A newly identified enhancer located at the 3' gene body of HER2 was reported to be the target locus of known



**Figure 4** 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.

HER2 regulator, TFAP2C.<sup>118</sup> Other epigenetic mechanisms, such as DNA methylation and histone modifications, also affect this process.

HER2 has been widely used in generating animal models for investigating cancer biology and therapeutic efficiency, especially in breast tumor models because of its powerful transformation-inducing ability.<sup>119–123</sup> A positive rate of 15%–20% in HER2 status (Overexpression/Amplification) (3+ by IHC or an amplified HER2 gene copy number by FISH)<sup>124</sup> generally identifies those women who might benefit from HER2 targeted therapies, such as monoclonal antibodies or tyrosine kinase inhibitors (TKIs). Such novel targeted treatments have greatly improved the prognoses of patients with HER2+ neoplasms.<sup>125,126</sup>

Breast cancer cells that express HER2 are more likely to progress to metastasis.<sup>127</sup> Progesterone and progesterone-induced paracrine signals likely induce migration in early may induce migration in early primary tumor cells and, in this way, activate mammary stem cells. This proposed impact is consistent with HER2-stimulated stem-cell qualities that have been reported.<sup>123</sup> Thus, HER2 testing is used to select patients appropriate for what is potentially resistant and expensive therapy.<sup>128</sup> In pursuit of heightened specificity of treatment, HER2 molecular analysis has become an integral part of the diagnostic breast cancer patient work-up.<sup>128</sup>

### Canonical Wnt/ $\beta$ -catenin signaling in breast cancer

Wnt proteins are a family of highly glycosylated, secreted proteins with pivotal roles in various developmental processes including embryonic induction, generation of cell polarity, and cell fate specification, as well as in maintaining adult tissue homeostasis.<sup>129–136</sup> The canonical Wnt/ $\beta$ -catenin signaling is initiated by the binding of the secreted Wnt proteins, which is palmitoylated by Porcupin, to both co-receptors Frizzled and low-density lipoprotein receptor-related protein 5 and 6 (LRP5/6).<sup>131,132,134,135</sup> Wnt–receptor interaction leads to recruitment of Axin and Dishelved proteins to the cell membrane and induces inhibition of glycogen synthase kinase (GSK)-3 $\beta$  protein, which is a negative regulator of the Wnt pathway driven by  $\beta$ -catenin to proteasomal degradation that is induced by phosphorylation.<sup>131,132,134,135</sup> Inhibition of GSK-3 $\beta$  leads to  $\beta$ -catenin accumulation in the cytoplasm, and its subsequent translocation into the nucleus to act as co-transcriptional activator together with CREB binding protein (CBP) and T-cell factor/lymphoid enhancing factor (TCF/LEF) transcription factors and regulating oncogenes, such as *MYC*, *CCND1* and other target genes (Fig. 5).<sup>131–138</sup>

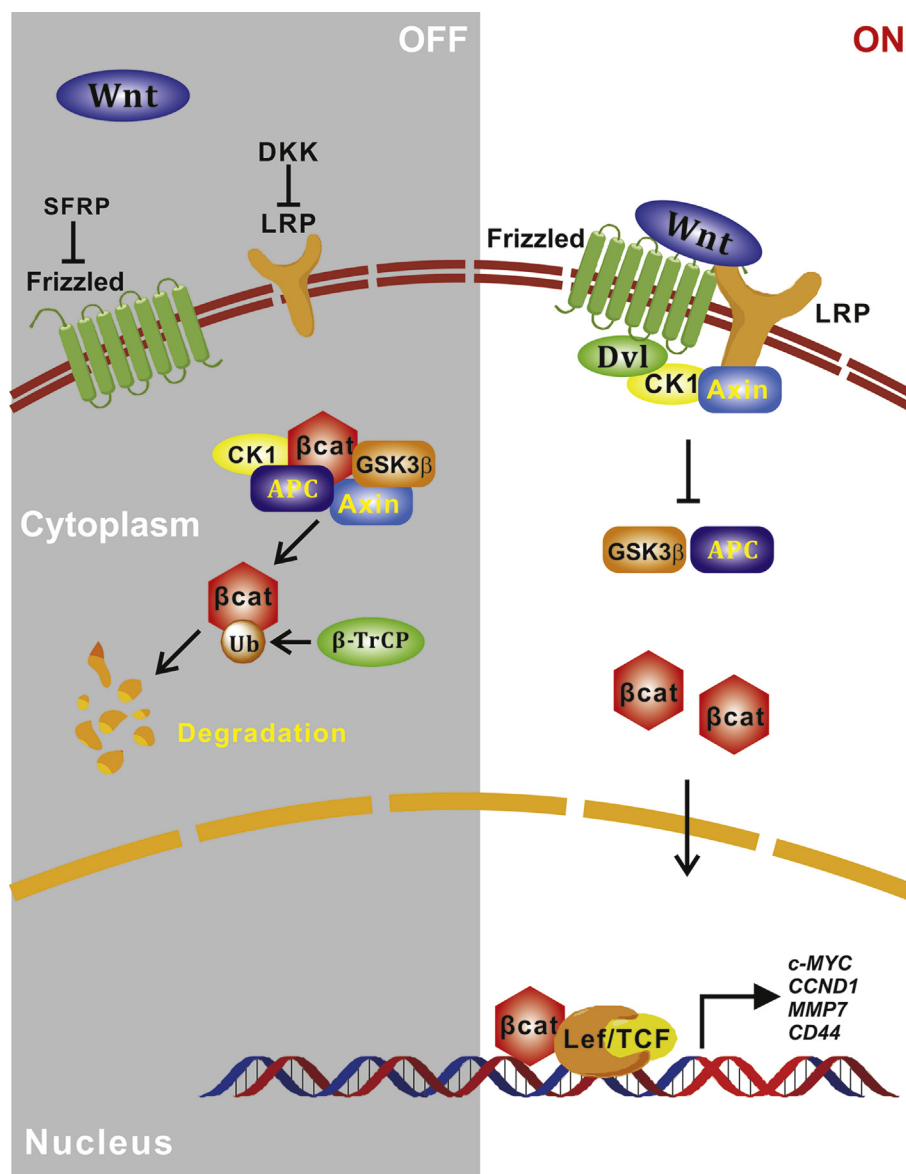
The first clue about the role of Wnt signaling in mammary gland development came from the identification of the first mammalian Wnt molecule, Wnt1, as MMTV-induced mouse mammary oncogenesis involved an insertion at Wnt1 locus (or called MMTV int1).<sup>139</sup> Soon after, Wnt3 was identified as another common insertion site for MMTV-induced mammary tumors in mouse.<sup>140,141</sup> Accordingly, mammary gland-specific expression of stabilized  $\beta$ -catenin has been shown to result in aggressive adenocarcinomas, consisting predominantly of glandular and undifferentiated cells.<sup>142</sup> Interestingly, while neither Wnt1 nor Wnt3 is highly

expressed in the adult mammary gland, other members of the Wnt family, such as Wnt-2, 4, 5a, 5b, 6, and 7, are expressed at various stages of mammary development.<sup>143–145</sup> Wnt-2, 5a, and 7b are strongly expressed in the virgin mouse mammary gland, but are downregulated during pregnancy, while pregnancy strongly induces the expression of Wnt-4, 5b, and 6.<sup>144</sup> Overexpression of Wnt-4 was shown to result in an increase in ductal branching,<sup>146</sup> while the mammary tissue of Wnt-4<sup>-/-</sup> mice exhibited significantly reduced ductal branching compared to their wild-type counterparts.<sup>147</sup> Mice lacking Lef-1 or Lrp6, and mice overexpressing the Wnt inhibitor Dickkopf all failed to develop an appropriate mammary bud.<sup>148–150</sup> Studies using the Wnt reporter mice (Axin2-LacZ) showed positive staining in nearly all branches of the mammary ductal system, and Wnt activity was detected in cells located in the basal layer of the mammary ducts, which has been suggested to be the MaSC niche.<sup>151,152</sup>

In breast cancer, Wnt signaling is likely constitutively activated through an autocrine mechanism. While mutations in Wnt signaling are not common in breast cancer, it has been shown that approximately 50% of clinical breast cancer cases exhibit high levels of stabilized  $\beta$ -catenin.<sup>153,154</sup> The positive regulator of Wnt signaling Dvl is amplified in 50% of breast cancers.<sup>155</sup> Conversely, a secreted Wnt inhibitor, Frizzled-related protein 1 (FRP1) was reportedly lost in 78% of malignant breast cancers,<sup>156</sup> and associated with poor prognosis.<sup>157</sup> Down regulation of Wnt inhibitor Dickkopf 1 (*DKK1*) suggests the importance of Wnt regulation in the metastatic process in breast cancer.<sup>158</sup> Furthermore, the expression of APC is lost in approximately 36–50% of breast cancers either by mutations, loss of heterozygosity, or hypermethylation.<sup>159,160</sup> The Wnt/ $\beta$ -catenin pathway was remarkably activated in basal-like breast tumors, and its nuclear localization was correlated with worse prognosis.<sup>161,162</sup> Not only does activated  $\beta$ -catenin promote triple-negative breast cancer, it also has a role in HER2-driven mammary tumors shown by *in vivo* data.<sup>163</sup> With many of Wnt ligands being able to promote the progression of breast tumors, the restoration of many Wnt inhibitors that has been silenced by mechanisms such as DNA methylation and miRNAs in tumors has effectively attenuated tumor growth.<sup>164–166</sup> Wnt pathway activation was shown to increase radiation resistance of progenitor cells in the mouse mammary gland and human breast cancer cell lines,<sup>167,168</sup> indicating that Wnt signaling is involved in resistance to current anticancer drugs potentially by regulating stem and progenitor cell populations.

Emerging evidence suggests that breast cancer initiation and maintenance may be regulated by a small population of cells within the tumor, either stem cells or cells that exhibit stem-like properties.<sup>169</sup> The expression of Wnt1 in human mammary epithelial cells increased stem cell self-renewal, resistance to apoptosis and failure to senescence.<sup>170</sup> Similarly, an expanded mammary stem cell pool was identified from a population of committed luminal progenitors in MMTV-Wnt1 mouse model,<sup>171</sup> indicating that Wnt-1 activation may induce the appearance of aberrant progenitor cells. It was also found there was a 6.4-fold expansion of MaSCs in premalignant MMTV-Wnt1 transgenic mammary glands.<sup>152</sup> The pre-neoplastic lesions and tumors of Wnt-1





**Figure 5 Canonical Wnt/β-catenin signaling pathway.** Canonical Wnt signaling plays significant roles in many biological and pathological processes such as mammary gland development and breast tumorigenesis. Wnt ligands bind with membrane receptors frizzled and LRP, attenuating the ubiquitination of β-catenin by β-TrCP. Accumulation of β-catenin allows for nucleus translocation and downstream transcriptional activation. Inhibitors of Wnt signaling such as DKCs and SFRPs function as tumor suppressors by contributing to the degradation of β-catenin, thus impeding the transcription of β-catenin-targeting oncogenes.

mice were shown to have expanded stem/progenitor cell populations.<sup>172</sup> Similar results were obtained from transgenic mice expressing β-catenin or c-myc, two downstream components of Wnt signaling, while mammary tumors in transgenic mice expressing Neu, H-Ras or polyoma middle T antigen did not exhibit a similar enrichment for MaSCs.<sup>172</sup> Conditional deletion of a single APC allele in either the mammary stem/progenitor population or luminal cells of lactating mice revealed that mammary tumors only developed when APC was deleted in mammary progenitor cells, but not in luminal cells, suggesting Wnt-induced tumorigenesis targets the stem/progenitor population.<sup>173</sup> Lastly, the inhibition of WNT1 was shown to alter the phenotype to CD44<sup>+</sup>CD24<sup>-</sup>ALDH1<sup>-</sup> and reduce tumor formation and cellular migration.<sup>174</sup> Furthermore, suppression of GSK3/β-

catenin signaling *via* the inhibitory activity of protein kinase D1 (PRKD1) was sufficient to reduce the stemness features of breast cancer cells.<sup>175</sup> Thus, Wnt signaling may play an important role in maintaining mammary stem cell properties.

### Other signaling pathways in breast cancer

Normal mammary stem cells and mammary development is controlled by a variety of hormones and signaling pathways.<sup>151</sup> In addition to the three pathways discussed above, many other pathways and their crosstalk play important roles in regulating normal mammary development, as well as in breast cancer development if they are dysregulated.

These include CDKs (Cyclin dependent kinase), Notch, SHH, PI3K/Akt/mTOR, and others.<sup>128</sup>

**Cyclin dependent kinases (CDKs):** Cell cycle progression is regulated by cyclins, CDKs, and CDK inhibitors (CDKIs).<sup>176</sup> All cancers activate the cell cycle to sustain their survival. The overexpression of cyclin D1 and cyclin E as well as the decreased expression of CDKI p27Kip1 were found in human breast cancer.<sup>177</sup> Cyclin D1 amplification is seen in nearly 60% of breast cancers. Furthermore, estrogen utilizes cyclin D1 to exert its mitogenic effects. It was reported that high tumor expression of cyclin D1 and overexpression of HER2 were associated with reduced recurrence-free survival and tamoxifen responsiveness.<sup>178</sup> The oral CDK4/6 inhibitor Palbociclib alone was shown to inhibit cell cycle progression ER-positive cell lines, including those with HER2 amplification, which were most sensitive to growth inhibition by palbociclib while nonluminal/basal subtypes were most resistant.<sup>91</sup> Thus, Palbociclib is a promising therapeutic in breast cancer, and has been recently approved as a combination with fulvestrant for the treatment of hormone receptor-positive, HER2-negative metastatic breast cancer.<sup>128,179</sup>

**Notch signaling:** Notch signaling is implicated in the pathogenesis of breast cancer and as such may represent a novel therapeutic target. Notch signaling consist of five Notch ligands, namely Delta-like (DLL) 1, 3, 4, and Jagged (JAG) 1, 2, which are single transmembrane proteins.<sup>180</sup> There are four Notch receptors which act through the same basic signaling pathway and is activated by binding of Notch ligand on one cell to the extracellular domain of a Notch receptor on another neighboring cell. The Notch ligand–receptor complex then undergoes several key proteolytic cleavages, yielding the Notch extracellular domain and the Notch intracellular domain (NICD). NICD acts as a transcriptional factor and regulates downstream target genes.<sup>180</sup> It was reported that high-level expression of Jag1 (Jag1<sup>High</sup>) and/or Notch1 (Notch1<sup>High</sup>) in tumors correlated with poor outcome and is an independent prognostic indicator in primary human breast cancers.<sup>181–183</sup> Mammosphere self-renewal was inhibited by Notch 4 blocking antibody or an inhibitor of the  $\gamma$ -secretase enzyme,<sup>184</sup> and the efficiency of DCIS-derived mammosphere production was significantly reduced when Notch signaling was inhibited.<sup>185</sup> In primary breast cancer and breast cancer cell line-derived tumorspheres, Notch 3 and Jag1 have emerged as key regulators of cancer stem cell renewal and hypoxia survival.<sup>186</sup> Furthermore, Notch signaling also interacts with HER2 signaling pathway, which is active in approximately 20% of breast cancers and associated with a more aggressive disease.

**Sonic Hedgehog (SHH) signaling:** SHH signaling plays a critical role in organizing cell growth and differentiation during embryonic tissue patterning,<sup>187,188</sup> and is important in mouse mammary gland development. Disruption of its downstream transcriptional targets, Patched homolog-1 (PTCH-1) or glioma-associated oncogene-2 (GLI-2) resulted in severe defects in ductal morphogenesis.<sup>189,190</sup> It was shown that disruptions of these genes also occurred in breast cancer,<sup>190,191</sup> suggesting a role for the SHH pathway in breast tumorigenesis. This data suggests that SHH activation may contribute to the relapse of breast cancer and may serve as a predictor of postoperative relapse since

higher expression of SHH, PTCH-1, GLI-1, and SMOH in breast cancer was shown to correlate with breast cancer invasiveness.<sup>191</sup>

**Breast tumor kinase (BRK):** BRK is a non-receptor tyrosine kinase identified in breast tumors,<sup>192</sup> and is overexpressed in more than 60% of breast cancer cases, but not in normal mammary glands or benign lesions.<sup>193,194</sup> Depletion of BRK in breast cancer cells was shown to impair EGFR-regulated signaling.<sup>195</sup> Activation of BRK by stably expressing BRK-Y447F significantly increased MAPK activity, cell proliferation and migration in breast cancer cells, while a decreased migration was observed in breast cancer cells depleted of BRK.<sup>196</sup> Thus, BRK may play an important role in promoting cell proliferation and migration in breast cancer cells.

**PI3K/AKT/mTOR pathway:** As discussed in the following section, oncogenic mutation of PI3K (i.e., PIK3CA) is a common one in human breast cancer that may lead to the dedifferentiation of luminal or basal mammary progenitor cells, allowing them to attain multi-lineage potential.<sup>197</sup> Hyperactivation of AKT and the subsequent hyperactivation of downstream mTOR may underlie resistance to endocrine therapies.<sup>198–200</sup> Clinically, activation of AKT is associated with a worse outcome among patients receiving endocrine therapy, with reduced clinical benefit in patients with positive expression of activated AKT.<sup>201</sup> It was reported that there was an inverse correlation between AKT activation and partial response rates.<sup>202</sup> Accordingly, the expression of phosphorylated S6 kinase (S6K), a downstream mediator of mTOR activation, can predict overall survival in patients with hormone receptor-positive breast cancer receiving adjuvant endocrine therapy.<sup>115</sup> Thus, mTOR inhibition may be able to restore anti-estrogen sensitivity.

## Cancer gene mutations in breast cancer

### BRCA1/2 mutations in breast cancer

Approximately 10%–20% breast cancer patients have at least one first-degree relatives affected with breast cancer.<sup>8,203</sup> Among them, up to 20% of women with a family history of breast cancer have a mutation in the breast cancer susceptibility genes 1 or 2 (*BRCA1* or *BRCA2*).<sup>204</sup> Thus, the significance of *BRCA1* and *BRCA2* in breast cancer can be told from their names.<sup>205,206</sup> The prevalence of germline *BRCA* mutations is relatively high in women of Ashkenazi Jewish ethnicity, where the risk is estimated to be 30%–35%.<sup>207–209</sup> In male breast cancer cases, up to 14% have a *BRCA2* mutation although 4.5% of Ashkenazi Jewish men presenting with breast cancer have a *BRCA1* mutation.<sup>210,211</sup> Moreover, among women with ovarian cancer, regardless of family history, about 15% are attributable to *BRCA* mutations,<sup>212</sup> while the proportion with a germline *BRCA* mutation may be as high as approximately 40% in Ashkenazi Jewish women with epithelial ovarian cancer.<sup>213</sup>

The *BRCA* proteins share a similar, and cooperative, tumor suppressing mechanism by repairing DNA damage through homology-directed repair (HDR), which inhibits tumorigenesis.<sup>206,214</sup> Thus, deletion mutations and/or loss of function in the *BRCA* genes lead to decreased DNA repair

efficiency and possibly give rise to the expansion of cancerous cells, elevating the risk of developing breast cancer by five to six fold.<sup>1,215</sup>

Even though *BRCA* mutations can be inherited and responsible for a certain proportion of familial cases, investigators have found no difference in the incidence of breast cancer in *BRCA* mutation carriers with or without intimate family history.<sup>216</sup> A recent study has shown that smoking also plays a minor role in increasing the likelihood of developing breast cancer, as well as other cancers for *BRCA*-mutation carriers.<sup>217</sup> These results will increase the accuracy of identifying the high-risk population and the efficiency of preventative measures.

For more than two decades, the prevalence of genetic variations of the *BRCA* genes in breast cancer and other cancers has been well-investigated.<sup>218–223</sup> Interpretations that are more comprehensive were made available with the advancement of data collection and analysis. Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA; <http://cimba.ccge.medschl.cam.ac.uk/>) has made significant contributions to the characterization of the *BRCA* landscape. A latest update report of the CIMBA dataset summarized a total of 1650 and 1731 unique mutations in *BRCA1* and *BRCA2* respectively.<sup>224</sup> Of all the types of *BRCA1* and *BRCA2* mutations, frameshift is the most common, mostly leading to the generation of premature stop codons and therefore decreasing the levels of mature RNAs and functional proteins. Enduring efforts made by researchers or organizations such as CIMBA demonstrate the ubiquitous perception of higher ratio of *BRCA* mutations in younger patients with more aggressive subgroups of breast cancer.<sup>223,225–227</sup> Nonetheless, the majority of these studies imply a comparable outcome among patients regardless of *BRCA* status, limiting the application of *BRCA* mutation status in prognosis prediction.

Clinical data suggest that *BRCA1* mutation-related breast cancers that do not overexpress ER or ERBB2 are related to the expression of the basal epithelial markers, which are already associated with ER/ERBB2-negative tumors.<sup>228</sup> While the cells are phenotypically basal-like, there is evidence that *BRCA-1* basal-like breast cancer cells as well as sporadic basal-like breast tumors are not originated from basal stem cells, but rather from luminal epithelial progenitors.<sup>229</sup> Cells with a potential for phenotypic plasticity do not necessarily reflect histology in tumor phenotypes.<sup>229</sup>

### Oncogenic mutations of *PIK3CA* in breast cancer

Phosphatidylinositol 3-kinase (PI3K) is divided into three classes (I–III) based on their structure and substrate specificity. Class I PI3K is further categorized into class IA and IB, in which Class IA PI3K is the class most closely implicated in cancer.<sup>230</sup> Structurally, PI3K is constituted of a p110 catalytic subunit and p85 regulatory subunit. There are three isoforms of p110, namely p110 $\alpha$  (encoded by *PIK3CA*), p110 $\beta$ , and p110 $\delta$ . While p110 $\delta$  is expressed exclusively in leukocytes, p110 $\alpha$  and p110 $\beta$  are ubiquitously expressed.<sup>230,231</sup> Conversely, human regulatory subunits p85 $\alpha$ , p85 $\beta$ , and p55 $\gamma$  are encoded by PI3K regulatory subunit 1 (*PIK3R1*), *PIK3R2*, and *PIK3R3*, respectively.<sup>231</sup>

PI3K signaling is initiated by the growth factor activated receptor tyrosine kinase, or RAS protein, though direct interaction with p85 or *via* adaptor proteins, resulting PI3K being recruited to the membrane.<sup>230,231</sup> Activated PI3K subsequently activates critical downstream mediators AKT and mTOR, leading to enhanced growth, anti-apoptosis, cell-cycle progression, and translation.<sup>230–232</sup>

The PI3K/AKT/mTOR pathway is the most frequently enhanced oncogenic pathway in breast cancer.<sup>230</sup> Among mechanisms of PI3K enhancement, *PIK3CA* mutations are most frequently (~30%) observed, along with protein loss of PTEN,<sup>230,233</sup> although somatic mutations of *PIK3CA* coding p110 $\alpha$  in various solid malignancies were first reported in 2004.<sup>234</sup> The majority of *PIK3CA* somatic mutations are located in the two “hot spots”, E542K or E545K in exon 9, and H1047R or H1047L in exon 20, both of which are gain-of-function mutations and have transforming capacity.<sup>230,234</sup> Interestingly, *PIK3CA* gene amplification was reported even before *PIK3CA* mutations were identified, and was found in various malignancies, including approximately 10% of cases of breast cancer.<sup>235,236</sup>

In addition to *PIK3CA* mutations, there are many other PI3K-enhancing mechanisms, such as *HER2* amplification, dysfunction of PTEN, and *AKT1* activating mutation. For example, *PIK3R1* mutations were found in breast cancer with much lower occurrence (~3%) as the *PIK3R1* gene product p85 $\alpha$  plays a tumor-suppressor role by stabilizing p110 $\alpha$ .<sup>38,230</sup> *AKT1* mutations (E17K) have been found in 1.4%–8% of breast cancers, especially in tumors expressing both ER and PR.<sup>237</sup> *PIK3CA* mutations and gain of copy number of *PIK3CA* and PTEN loss and *PTEN* mutations have also been reported to coexist, although *PIK3CA*, *PTEN*, *AKT1*, and *PIK3R1* mutations are reported to be mutually exclusive.<sup>230,237,238</sup>

Oncogenic *PIK3CA* mutations are thought to cause dedifferentiation of luminal or basal mammary progenitor cells, allowing them to attain multi-lineage potential.<sup>197</sup> Mutations that upregulate the PI3K pathway in ER-positive breast cancers, such as downregulation of PTEN, overexpression of *HER2* or IGF-1R, or the activation of mutant *AKT1*, can lead to the acquired resistance to hormone therapies in ER-positive tumors.<sup>233</sup> Overall, *PIK3CA* mutations are most likely found in luminal-type (HR-positive/*HER2*-negative) tumors, in particular those with markers indicating less aggressive tumor characteristics.<sup>230</sup> Furthermore, it was reported that treatments with PI3K inhibitors in ER-positive, *PIK3CA*-mutant breast cancers had to be continuous to achieve optimal therapeutic effects, and to overcome proliferative rebound and antiestrogen resistance; and yet the occurrence of such resistance observed in one third of patients in this subtype and resulted in an exceptionally poor prognosis.<sup>239</sup> Nonetheless, it remains to be determined whether *PIK3CA* mutations are valid prognostic or predictive biomarkers for the clinical management of breast cancer patients.

### Other gene mutations in breast cancer

Mutations of the *BRCA1* and *BRCA2* genes do not explain the occurrence of breast cancer in every breast cancer prone family.<sup>240–242</sup> In fact, several breast cancer

susceptibility genes, many of which are associated with rare genetic syndromes, have been identified and may account for less than 1% of all hereditary breast cancers.<sup>240–243</sup> These gene mutations are much less common and those representative of this group of genes include the following:

**ATM and ataxia telangiectasia:** The *ATM* gene product participates in damaged DNA repair. Most ataxia telangiectasia patients do not survive to an age at which breast cancer generally occurs. The risk of ataxia-telangiectasia carriers to develop breast cancer is estimated to be 11% by the age of 50 and 30% by the age of 70.<sup>244</sup>

**TP53 and Li-Fraumeni syndrome:** Inactivating mutations in the *TP53* gene have been found in many cancer types including breast cancer.<sup>245</sup> Li-Fraumeni syndrome is an autosomal dominant disorder, caused by germline mutations in the *TP53* gene, leading to an increased risk of osteosarcomas, leukemia, brain tumors, adrenocortical carcinomas, and breast cancers.<sup>246</sup> The risk of developing breast cancer before age 45 is 18-fold higher for affected females, as compared to the general population.<sup>241</sup> While germline mutations in the *TP53* gene may account for <1% of breast cancer cases, somatic mutations in the *TP53* gene are reported in 19–57% of human breast cancers<sup>241</sup> and LOH is found in 30–42%.<sup>241</sup>

**PTEN and Cowden syndrome:** Cowden syndrome is an autosomal dominant disorder, characterized by the development of hamartomas and benign tumors.<sup>241</sup> Mutations in the *PTEN* gene are present in 80% of Cowden syndrome families.<sup>247</sup> Truncating *PTEN* mutations in Cowden syndrome families cause a 25–50% lifetime breast cancer risk in women.<sup>241</sup> In sporadic breast cancer patients, germline and somatic mutations in the *PTEN* gene are rare.

**STK11 or LKB1 and Peutz-Jeghers syndrome:** Peutz-Jeghers syndrome is caused by truncating germline mutations in the *LKB1* gene and is an autosomal dominant disorder characterized by hamartomatous polyps in the small bowel and pigmented macules of the buccal mucosa, lips, fingers, and toes.<sup>241,248–250</sup>

**PALB2 (Partner and Localizer of BRCA2):** *PALB2* was originally identified as a *BRCA2*-interacting protein but subsequently also shown to interact with *BRCA1*.<sup>251,252</sup> Biallelic germline loss-of-function mutations in *PALB2* (also known as *FANCN*) cause Fanconi's anemia, whereas monoallelic loss-of-function mutations are associated with an increased risk of breast cancer and pancreatic cancer.<sup>253</sup> Loss-of-function mutations in *PALB2* are an important cause of hereditary breast cancer, with respect both to the frequency of cancer-predisposing mutations and to the risk associated with them.<sup>254–256</sup>

**CHEK2:** Checkpoint kinase 2 (*CHEK2*) is a serine/threonine kinase, which is activated upon DNA damage and plays an important role in governing DNA repair, cell cycle arrest or apoptosis in response to the initial damage. Loss of kinase function due to mutation is correlated with several types of cancer, mainly breast cancer.<sup>257</sup>

**CDH1:** *CDH1* encodes for the E-cadherin protein. Hereditary diffuse gastric cancer is an autosomal dominant inherited disease associated of *CDH1* germline mutations. While the affected individuals develop a rare type of stomach cancer, invasive lobular breast cancer is the second most frequent type of neoplasia.<sup>258,259</sup>

## Epigenetics and non-coding RNAs in breast cancer

The important role of epigenetic changes such as aberrant DNA methylation and histone modification in cancer causation, progression and treatment has been increasingly recognized and exploited, as the enzymatic processes that control the epigenome present new opportunities for developing therapeutic strategies to target transcriptional abnormalities that are inherent to the cancer epigenome.<sup>260,261</sup> In addition, epigenetic factors may provide a mechanism for the development of cancer cell heterogeneity. It was reported that the analysis of DNA methylation and gene expression identifies conserved, reproducible patterns of association in three main breast cancer cohorts, which were related to two main branches of expressed qualities, tumor infiltrating immune cell signatures and ER signaling.<sup>262</sup> The significant demethylation in the binding sites at the enhancers of three well-studied transcriptional factors, ER $\alpha$ , FOCA1, and GATA has been detected in ER + breast cancers in comparison to normal breast tissue.<sup>262–264</sup> The elevated expression of these genes was also seen to be elevated in Luminal A and Luminal B types in contrast to Normal- and Basal-like breast cancers strongly linking this cluster of transcription factors to estrogen signaling.<sup>262</sup> DNA methylation at the enhancers of ER $\alpha$  binding sites may also be a root cause of resistance to anti-estrogen treatments like tamoxifen for ER + breast cancers.<sup>97,265–267</sup>

Recent rapid advances in large-scale whole genome sequencing and RNA sequencing techniques, in combinations with bioinformatics analyses have profoundly changed our understanding about human genome. Surprisingly, comprehensive global transcriptomic analyses indicated that up to 80% of the human genome while <2% of them encode proteins.<sup>268–271</sup> Thus, the vast majority of mammalian transcriptome encompass noncoding RNAs (ncRNAs) that play a variety of important regulatory roles in gene expression and other biological processes.<sup>271,272</sup> The ncRNAs are divided into two categories according to the size and structural or regulatory properties. The ncRNAs >200 nucleotides are called long noncoding RNAs or lncRNAs, whereas ncRNAs <200 nt include microRNAs (or miRNAs), small nucleolar RNAs (or snoRNAs), and piwi RNAs (or piRNAs).<sup>272–274</sup> The miRNAs is the best studied short noncoding RNAs (sncRNAs), being extensively characterized for their biogenesis, function and importance in tumorigenesis.<sup>270–272</sup> On the other hand, lncRNAs have emerged as key regulators of developmental processes, including mammary gland development.<sup>271,272</sup> In fact, lncRNA dysregulation is implicated in the development of various cancers, including breast cancer.<sup>272</sup>

A recent genome-wide transcriptional survey was carried out to explore the lncRNA landscape across 995 breast tissue samples was recently performed.<sup>275</sup> A total of 215 lncRNAs were found aberrantly expressed in breast tumors, as compared to normal samples.<sup>275</sup> On the basis of lncRNA expression, unsupervised hierarchical clustering of breast tumors identified four breast cancer subgroups that correlate tightly with PAM50-defined mRNA-based subtypes.<sup>275</sup> Further analysis of the co-expression of lncRNA genes and



protein-coding genes revealed a correlation, on one hand, between luminal A-specific lncRNAs and the activation of PI3K, FGF and TGF- $\beta$  pathways, and on the other hand, between basal-like-specific lncRNAs and the activation of EGFR-dependent pathways and of the epithelial-to-mesenchymal transition, suggesting a wide range of biological functions associated with lncRNAs in breast cancer.<sup>275</sup>

Recent studies have shown that ncRNAs are key players in the initiation and progression of cancer, and epigenetic mechanisms are deeply involved in their dysregulation. A growing list of miRNA genes aberrantly methylated in cancer, suggesting that many miRNAs may act as tumor suppressors or oncogenes.<sup>261</sup> In addition, it has been shown that dysregulation of lncRNAs plays critical roles in tumorigenesis.<sup>261</sup> Since ncRNAs are involved in regulating gene expression through interaction with epigenetic modifiers, their dysregulation may cause epigenetic alterations in cancer. Dissection of the interrelationships between ncRNAs and epigenetic alterations has the potential to reveal novel approaches to the diagnosis and treatment of cancer.<sup>261</sup> It has been shown that lncRNAs can impact a variety of factors through mediated regulation of protein coding RNAs by acting cis and/or trans to guide epigenetic modifier complexes to targeted sites and protein stability, as well as by affecting miRNA binding capabilities.<sup>276,277</sup> LncRNAs, and other noncoding RNAs such as miRNAs, work in conjunction with RNA-binding proteins (RBPs), to alter both co- and post-transcriptional gene regulation.<sup>278</sup> There are many cases in which, through epigenetic modifications, lncRNAs act as gene suppressors though lncRNAs may also act as gene activators.<sup>279,280</sup> MANCR (mitotically-associated noncoding RNA) is a lncRNA of particular interest, especially in its proposed association to TNBC, in the current search for therapeutic targets for its noted upregulation in breast cancer cells.<sup>281</sup>

Consistent with the genomic instability associated with TNBC, the mutation rate in both coding and noncoding regions were significantly higher in ER-negative/HER2-negative tumors.<sup>282</sup> Substitution errors are the most frequent aberrations in noncoding regulatory regions, namely the promoters of cancer-associated genes including OBSCN and TP53. Additionally, identified genetic variations in promoters, introns, and other such regulatory noncoding regions may impact the phosphorylation, protein-protein interaction, and regulation of cancer-associated genes including ATM/ATR, FGFR1, FOXA1, IGF1R, NF1, NOTCH2, and TOP2A.<sup>282</sup> It has been reported that the hypomethylation phenotype is observed at a higher rate in many lncRNAs in breast cancer and other cancers as opposed to the well-studied phenomenon of CpG island hypermethylation phenotype (CIMP) in tumors.<sup>262</sup> The hypomethylation of lncRNA EPIC1 upregulates its expression, and EPIC1 overexpression is related to poor survival outcomes in independent patient cohorts, in particular, for luminal B breast cancer, and was shown to promote tumorigenesis through interacting directly with MYC to increase the occupation of MYC target genes and promote cell-cycle progression.<sup>265</sup>

The impacts of lncRNA epigenetic alterations can be studied using a repurposing of HM450 probes to analyze lncRNA promoters and comparing the lncRNA DNA

methylation profile of lncRNA promoters to an existing database.<sup>265,283</sup> In fact, several studies identified lncRNA copy-number alterations and expression alterations, on account of which there is a point of reference for looking into methods for identifying and understanding potentially relevant lncRNAs.<sup>265,277,284</sup> A recent pan-cancer analysis of S-phase enriched lncRNAs using nascent RNA capture assays has identified hundreds of potential biomarkers and oncogenic drivers, contributing to the process of illuminating the role of lncRNAs in S-phase regulation.<sup>277</sup> Nonetheless, while herculean efforts have been in place to identify genetic driver mutations for breast cancer, the realm of noncoding RNA sequences and epigenetic factors remain largely unexplored by comparison.

## Molecular basis of triple-negative breast cancer (TNBC)

Triple-negative breast cancer (TNBC) is broadly defined as tumors that lack expression of the estrogen receptor (ER), progesterone receptor (PR), and HER2.<sup>285–287</sup> TNBC accounts for approximately 20% of breast cancers and is more commonly diagnosed in women younger than 40 years, as well as in African-American women.<sup>288</sup> Genetically, <20% of patients with TNBC harbor a breast cancer gene (BRCA) mutation, particularly in *BRCA1*.<sup>289</sup> Pathologically, TNBC is usually high grade and commonly infiltrating ductal carcinoma exhibiting geographic necrosis.<sup>290</sup> TNBC Patients usually have a poorer outcome compared with those with other breast cancer subtypes owing to an inherently aggressive clinical behavior and a lack of effective targeted therapies.<sup>286,291</sup> The diagnosis of TNBC relies on the accurate determination of ER and PR protein levels by immunohistochemistry (IHC) and of HER2 by IHC and/or fluorescence in situ hybridization (FISH).<sup>286,287</sup> Such accurate assessment is crucial to avoid false diagnosis of ER-negative and/or HER2-negative disease in patients that would be benefited from endocrine therapy and/or HER2-targeted drugs.<sup>285,286</sup> TNBC clinical phenotype usually consists of the basal-like molecular subtype, although TNBC and basal-like breast cancers are not synonymous and yet there is substantial heterogeneity within TNBCs.

The molecular landscape of TNBC is complex and remains to be fully understood, largely due to the heterogeneous genetic and clinical features. An extensive IHC staining study of 172 TNBC samples revealed that only 71% of TNBCs were assigned the basal subtype.<sup>292</sup> In a converse analysis of 160 tumors, in which subtype was identified and correlated with IHC staining, 77% of basal tumors were triple-negative by IHC.<sup>292</sup> A single-cell sequencing study was attempted to dissect TNBC-specific tumor heterogeneity.<sup>293</sup> On the basis of gene expression profiles, six TNBC subtypes were identified, including two basal-like-related subgroups (basal-like 1 and 2, or BL1, and BL2), two mesenchymal-related subgroups (mesenchymal or M, and mesenchymal stem-like, or MSL), one immunomodulatory subgroup (IM), and one luminal androgen receptor group (LAR).<sup>286,293</sup> Such TNBC subtypes were further shown to closely link to histological types, IM tumors overlapped with medullary breast cancer, M and MSL tumors with metaplastic breast cancer, and LAR tumors with apocrine

tumors.<sup>286,293</sup> Interestingly, a separate study involving genomic and transcriptomic analyses of 2000 breast tumors identified 10 integrative clusters (IntClust) of breast cancers.<sup>294</sup> It was shown that basal-like tumors were heterogeneously distributed among different groups, with IntClust 4 and 10 accounting for 80% of them.<sup>286,294</sup> Cancers classified as IntClust 4 had extensive lymphocytic infiltration with a strong immune and inflammatory signature and few copy-number aberrations ('CNA-devoid' subgroup) and patients within this subgroup had a favorable outcome,<sup>286,294</sup> further highlighting the clinical relevance of breast cancer heterogeneity. Conversely, the basal-like tumors within the IntClust 10 subtype had high genomic instability with major chromosomal aberrations, such as chromosome 5 loss, 8q gain, 10p gain or 12p gain.<sup>286,294</sup>

A direct comparison between TNBC subtypes and intrinsic molecular (PAM50) subtypes also revealed that most TNBCs were classified as basal-like (80.6%), followed by HER2-enriched (10.2%), normal-like (4.7%), luminal B (3.5%) and luminal A (1.1%) using PAM50 subtyping.<sup>295</sup> A direct overlap comparison between the PAM50 classification and the TNBC subtypes indicated that the majority of the TNBC subtypes were classified as basal-like tumors by PAM50 analysis (BL1 at 99%, BL2 at 95%, IM at 84% and M at 97%), with the exception of MSL and LAR.<sup>286,295</sup> Approximately 50% of MSL TNBCs were classified as basal-like, 28% as normal-like and 14% as luminal B tumors.<sup>295</sup> Furthermore, tumors within the LAR subtype were mainly classified as HER2-enriched (74%) or luminal B (14%).<sup>286,295</sup> Nonetheless, a recent molecular profiling analysis of TNBCs also identified four stable subtypes, luminal androgen receptor, mesenchymal, basal-like immunosuppressed, and basal-like immune-activated.<sup>296</sup>

While it is critical to identify molecular and genetic landscapes of TNBC at genomewide level, it is also important to delineate the contributing roles of individual genes and/or pathways in TNBCs, as those genes and/or pathways may serve as potentially actionable targets for TNBC treatments.<sup>286,297,298</sup> As forkhead box O (FOXO) transcription factors have a recognized role in tumor development and progression, it was shown that FOXO3a expression was highly expressed in TNBC tumors with negative clinical and pathological features, including lymph node metastasis and perineural invasion, and correlated with poor disease-free survival.<sup>299</sup>

Genome-wide mutational landscape analyses indicate that TNBC tumors on average carry 1.68 somatic mutations per Mb of coding regions and harbor ~60 somatic mutations in per tumor.<sup>38,300</sup> However, the mutation burden is not uniform; and some TNBCs may have a high mutation burden of >4.68 somatic mutations per Mb.<sup>38,286,300</sup> The overall consensus is that TNBCs have a frequent occurrence of multiple copy-number aberrations involving genes that lead to alterations in multiple signal pathways, which include the mutations/deletions of *BRCA1/2* in the DNA repair pathway; the mutations and/or amplifications of *PIK3CA* and *AKT3* and deletions/mutations of *PTEN*, *TSC1* and *INPP4B* of the PI3K/mTOR pathway; the amplifications of *FGFR1*, *EGFR* and *IGF1R*, mutations of *ERBB2*, *ERBB3* and *ERBB4*, and amplifications/mutations of *BRAF*, *KRAS*, and *HRAS* and the deletion of *DUSP4* of the RAS/RAF/MEK pathway; the deletions of *RB1* and amplifications of *CDK6*,

*CCND1* and *CCND2* of the cell-cycle checkpoints; the amplification of *JAK2* in the JAK/STAT pathway; along with androgen receptor pathway, Notch pathway, JNK/AP-1 pathway, and HIF1- $\alpha$ /ARNT pathway.<sup>38,286,287,294,297,298,300</sup> Many of these alterations may serve as novel targets for personalized targeted therapies in TNBCs. As nearly 90% of chemo-resistant TNBCs contain alterations in many of these pathways, some targeted agents such as PARP inhibitors, PI3K inhibitors, MEK inhibitors, heat shock protein 90 (HSP 90) inhibitors and histone deacetylase (HDAC) inhibitors, and BET bromodomain inhibitors, as well as immune checkpoint inhibitors, are currently under clinical investigation.<sup>286,287,297,298,301–304</sup>

## Tumor heterogeneity and evolution of breast cancer

Breast cancer tumor heterogeneity is one of the hallmarks of malignancy, which includes intertumor heterogeneity observed in breast cancers from different individuals and intratumor heterogeneity caused by the presence of heterogeneous cell populations within an individual tumor.<sup>23,305–308</sup> Breast cancer intratumor heterogeneity is the main hurdle in the development of effective treatments and personalized medicine.<sup>306–312</sup> The intratumor heterogeneity was first described by Rudolf Carl Virchow, one of the founders of modern pathology, in mid and late 19th century.<sup>313</sup> For quite a while, breast cancer phenotypic heterogeneity was used to classify breast cancers based on histological types.<sup>314</sup> The clinical implications of tumor heterogeneity were well-recognized early in the process, and breast cancer was one of the first solid tumor types, in which the clinical and treatment implications of heterogeneity for cellular phenotypes were established by analyzing the expression of the estrogen receptors.<sup>315</sup> With the rapid advances in molecular biology and genomics techniques, intratumor heterogeneity at the functional, genetic, and cellular levels has begun to be appreciated, and the identification of intrinsic molecular subtypes based on global gene expression profiling studies in breast cancer was pioneered with a rather rapid translation of this knowledge into clinical management of breast cancer.<sup>34,35,311,316</sup>

Breast cancer intertumor heterogeneity is best illustrated by clinical staging of the disease based on physical examination and imaging findings, while the morphologic heterogeneity of breast carcinoma constitutes the basis for the histopathologic classification of breast cancer.<sup>307</sup> Clinical grade of breast cancer is also highly representative of tumor heterogeneity, which is a reliable prognostic factor. However, breast cancers of different grades exhibit distinct profiles by proteomic, genomic and transcriptomic analyses, which serve as the basis to classify breast cancer into the five major intrinsic molecular subtypes with prognostic and therapy implications.<sup>286,297,298,301–304</sup> Furthermore, biomarker heterogeneity in breast cancers, especially the expression or lack of expression of ER, PR and HER2, is recognized as well-established prognostic and predictive factors as their expression in breast cancers is critical in guiding patient treatment. Nonetheless, numerous biomarkers have been investigated in breast cancer for

potential diagnostic, prognostic, and therapeutic implications.<sup>307,308,310,311</sup>

Breast cancer intratumor heterogeneity is likely caused by the phenotypic plasticity, clonal evolution of cancer stem cells and epithelial–mesenchymal transition (EMT) driven by epigenetic, genetic, and microenvironmental alterations in breast cancers.<sup>23,305–311</sup> Intratumor morphologic heterogeneity is usually appreciated as variability in different areas of tumor (or spatial heterogeneity) and as tumor progression over time (or temporal heterogeneity).<sup>307,308</sup> Morphologically distinct areas within individual tumors may indicate clonal evolution with specific genetic aberrations, while temporal heterogeneity may include evolution of an invasive tumor over time or in response to therapy, development of metastatic disease and progression from in situ to invasive carcinoma.<sup>301,307,317–320</sup>

The clonal evolution of genetically heterogeneous tumor initiating cells or cancer stem cells may contribute significantly to intratumor heterogeneity of breast cancer, even though the establishment of tumor heterogeneity has been traditionally explained by the cancer stem cell hypothesis<sup>23,305,321</sup> or the clonal evolution/selection model.<sup>322</sup> Both hypotheses propose that tumors originate from single cells that have acquired multiple molecular alterations and developed indefinite proliferative potential under optimal microenvironment to form a cancer.<sup>323,324</sup> However, the cancer stem cell hypothesis believes heterogeneity is caused by aberrant differentiation programs at an assumption of the existence of a hierarchical organization of cancer cells.<sup>323,324</sup> On the other hand, the clonal evolution hypothesis attributes intra-tumor heterogeneity to speciation by natural selection that does not rely on a hierarchical model.<sup>323,324</sup> Furthermore, the cancer stem cell hypothesis speculates that only a small fraction of stem cells are responsible for tumor progression and inherently therapy-resistant.<sup>323,324</sup> In the clonal evolution model, tumor progression and resistance to therapy should follow Darwinian evolutionary rules, in which the emergence of clones able to progress or be resistant to a therapy should depend on the size of cell population, gene mutation rate, cell proliferation rate, and selective pressures from the microenvironment and/or external selective pressures.<sup>323,324</sup> While each hypothesis has its pros and cons, increasing evidence suggests that these two hypotheses may not be mutually exclusive but rather complementary.<sup>324,325</sup> Nonetheless, given that fact that a high degree of phenotypic and genetic intratumor heterogeneity exists in breast tumors, the molecular, genetic and epigenetic mechanisms underlying intratumor heterogeneity remain to be fully understood.<sup>23,306,309</sup>

The tumor microenvironment may also contribute significantly to tumor heterogeneity as the tumor microenvironment includes not only cancer cells but also, immune cells, inflammatory cells, vascular cells, lymphatic cells, fibroblasts, and fibrous tissue, all of which impact the cancer's response to therapy.<sup>304,305,326,327</sup> Thus, a comprehensive assessment of individual patient tumor microenvironment may be beneficial for personalized medicine. It was shown, by altering their own immunogenicity as well as by implementing immunosuppressive responses in the tumor microenvironment, cancer cells can evade destruction by host immune system and continue to proliferate.<sup>328</sup> Thus, the recruitment of these normal cells

into the tumor microenvironment coupled with the acquisition of the self-perpetuating qualities in cancer cells may play a major role in sustained tumor development.<sup>327,329</sup> As the PD-1/PD-L1 is the immune checkpoint pathway and plays an important role in immunosuppression in the tumor microenvironment, it has been suggested that antibody inhibition in this pathway may bolster the natural immune system and be able to combat cancer cells.<sup>329</sup>

## Breast cancer metastasis

Cancer metastasis is a complex and yet inefficient process, which requires the regulation of a number of biological steps prior to the presentation of overt disease.<sup>330–332</sup> While the detailed mechanisms underlying cancer metastasis are far from understood, the metastatic cascade likely involves sequential events of tumor cell dissociation, neoangiogenesis of the primary tumor, intravasation, survival and diffusion through the lymphatic and systemic circulation, adhesion to target tissues, extravasation, establishment of metastatic foci at the tissue parenchyma, and a final manifestation of clinically apparent metastasis at the secondary site.<sup>330–332</sup>

Like many other solid tumors, breast cancer starts as a local disease, but can spread to the lymph nodes and distant organs, namely metastatic breast cancer (MBC, also known as advanced or stage IV breast cancers).<sup>331</sup> Breast cancer metastasis accounts for the majority of deaths from breast cancer. In fact, 10%–15% of breast cancer patients eventually develop distant metastases within three years after the initial detection of the primary tumor.<sup>331</sup> However, it is not unusual to see the manifestation of micro-metastases at distant sites >10 years after the initial diagnosis. Thus, patients with breast cancer are at risk of developing metastasis during their entire lifetime.<sup>331,333</sup> One salient feature of cancer metastasis is that many tumor types tend to preferentially colonize in certain tissues or organs. Breast cancers exhibit an organ-specific pattern of dissemination and preferentially metastasize to bone (occurrence rate at 47%–60%), the liver (occurrence rate at 19%–20%), lung (occurrence rate at 16%–34%) and brain (occurrence rate at 10%–16%).<sup>330–332,334–338</sup>

As for its primary tumor, metastatic breast cancer is highly heterogeneous, rendering it difficult to assess risk factors for metastasis and to find effective treatments of this disease.<sup>331–333,339</sup> A recent study examined the genomic evolution of breast cancer metastasis and relapse of the 299 samples from 170 patients with locally relapsed or metastatic breast cancer, and found that metastasis or relapse disseminated late from primary tumors, and continued to acquire mutations.<sup>340</sup> As a result, most distant metastases acquired driver mutations were not seen in the primary tumors, drawing from a wider repertoire of cancer genes than early drivers, many of which include a number of clinically actionable alterations and mutations inactivating SWI-SNF and JAK2-STAT3 pathways.<sup>340</sup> Thus, detection of breast cancer metastasis at the earliest stage is important for the management and prediction of breast cancer progression. Emerging techniques using the analysis of circulating tumor cells show promising results in predicting and identifying the early stages of breast cancer metastasis in patients.<sup>333</sup>

Despite intense efforts, our understanding of the underlying mechanisms of metastatic cancer in general has progressed minimally.<sup>332,341</sup> Over a century ago, the English surgeon Stephen Paget developed the seminal “seed and soil” concept, largely based on the observations that breast cancer preferentially spread to the liver but not the spleen.<sup>342</sup> The “seed and soil” hypothesis emphasizes that the selection of a site for secondary tumor development is not only made by the tumor cell (or the “seed”), but also influenced by the properties of the target organ (or “soil”).<sup>342</sup> Emerging evidence suggests that the relationship between disseminating tumor cells (DTCs) and the micro-environment of the target tissues/organs they colonize is the cornerstone of metastasis and plays a pivotal role in determining whether DTCs can survive and grow to form metastatic tumors.<sup>331,333,339,342</sup>

There are currently two prevailing and competitive models of metastasis, the early dissemination model, which hypothesizes that cancer cells with metastatic potential are seeded early in tumor formation and lay dormant for a period of time, and the late dissemination model, which suggests that metastasis may arise as a result of Darwinian selection during multistep tumor progression and the metastatic cells arise out of later stage tumor heterogeneity that produces more invasive, mesenchymal-like cancer cells.<sup>332,341,343,344</sup> The late dissemination model is seemingly supported by the fact that a successful metastatic cell must dissociate from the primary tumor, invade the surrounding tissues and penetrate the local vasculature, survive within the circulation, and be immobilized in the capillaries of target tissues/organs where it can extravasate to enter, survive and adapt to a novel and potentially inhospitable microenvironment.<sup>332,342,345</sup>

Extensive genomic studies have revealed that primary tumors and metastases of varied cancer types were closely related from a genetic standpoint, but failed to uncover genetic changes that are uniquely associated with progression to metastasis.<sup>346–350</sup> These results suggest that the metastasis seeding cells may originate from a subpopulation within the primary tumor and possess the genetic alterations required for both primary and metastatic tumor growth.<sup>332</sup> Such subpopulation must therefore rely on epigenetic and posttranscriptional programs superimposed on their genetic mutations to complete the metastatic process.<sup>332</sup>

Alternative to the clonal evolution model is that a small subpopulation of cells, or commonly referred to as cancer stem cells (CSCs), at the apex of the tumorigenic hierarchy may retain pluripotency and give rise to heterogeneous, differentiated progeny while maintaining its own numbers.<sup>332</sup> Given the fact that CSCs display tumor-initiating properties, it is conceivable that CSCs may also have the ability to disseminate and seed metastases, serving as metastasis-initiating cells (MICs), although cells that initiate metastatic growth have been difficult to characterize, and no hard evidence exists to suggest that MICs are distinct from CSCs.<sup>332,351–354</sup> Nonetheless, the CSC model should be compatible with the early dissemination hypothesis. It has been speculated that the latent phase of breast tumorigenesis may include the spread of breast cancer cells at an early, pre-symptomatic stage of primary tumor development when the disseminated cancer cells

remain undetected in bone marrow for years before presenting clinically identifiable features.<sup>123,355</sup> Dormant cancer cells with stem-cell properties in bone marrow can be activated in response to injury induced by chemotherapy to promote new tumor formation.<sup>60</sup> Furthermore, it is suggested that circulating tumor cells (CTCs) in the blood may play an active role of carcinoma metastasis, and the presence of CTCs correlates to poor patient prognosis and an increase in metastatic sites in patient data.<sup>356</sup> Nonetheless, strong evidence to draw a mechanistic connection between CTCs and tumor metastasis is lacking clinically, although it was shown in mouse models that metastasis-initiating cells (MICs) may exist in breast cancer CTCs.<sup>356</sup>

It has long been recognized that epithelial-to-mesenchymal transition (EMT) is an important cellular feature of metastatic cancer cells.<sup>357–359</sup> EMT is a critical pathway in the mesenchymal movement of single migratory cells, where the cancer cells can undergo changes from an epithelial phenotype to a mesenchymal-like phenotype.<sup>332</sup> The process is reversible, allowing epithelial cells, such as metastatic cancer cells that have undergone EMT to regain their epithelial features through mesenchymal-to-epithelial transition (MET).<sup>357–359</sup> It was shown that the EMT cells, while not found at secondary cancer sites in lineage-tracing studies, may be able to give neighboring non-EMT cells access to the secondary site thus influencing metastasis.<sup>360</sup> Furthermore, EMT process involves a number of cell functions which contribute to the imposition of mesenchymal traits on epithelial cells and thus can play a role in metastasis.<sup>361,362</sup> As EMT is well associated with cancer metastasis, tumor heterogeneity, and consequent drug resistance, the intersection between EMT regulation and hypoxia in the tumor microenvironment have been recently investigated.<sup>360,361,363</sup> MET may precede seeding at a secondary site in CTCs lodged in a capillary, which provides an advantage at this stage as they need no EMT to MET reprogramming to proliferate in capillaries or within secondary tissues.<sup>332</sup> Nonetheless, recent studies on pancreatic carcinoma models refuted EMT as a prerequisite for metastasis, suggesting instead that EMT may be more relevant to resistance to therapy.<sup>364,365</sup> Thus, it remains to be determined whether EMT is required and necessary for breast cancer metastasis.

Given the deadly consequence of metastatic breast cancer, it is imperative to detect metastasis at its earliest stage.<sup>331,366</sup> Importantly, the detection of biomarkers is usually necessary steps to determine treatment options.<sup>366</sup> The American Society of Clinical Oncology recommends 13 categories of breast tumor markers that showed evidence of clinical utility and were recommended for use in practice including ER, PR, HERs, carcinoembryonic antigen (CEA), CA 15-3, CA 27.29, urokinase plasminogen activator (UPA), plasminogen activator inhibitor 1 (PAI), and certain multi-parameter gene expression assays.<sup>366</sup> Among these biomarkers HER2 has attracted much attention as a possible prognostic marker.<sup>331</sup> With the rapid advances in genomic sequencing technologies, it is expected that new diagnostic and prognostic markers of breast cancer metastasis will be identified.<sup>367,368</sup> These markers will be likely in multi-gene panel formats. Furthermore, the drastic improvements over detection sensitivity and throughput of whole genome sequencing technologies will enable us to reliably detect



genomic changes in circulating tumor cells.<sup>369–373</sup> Till then the full potential of personalized precision medicine will be realized in the clinical management of breast cancers.

## Concluding remarks

In this review, we provide a comprehensive survey on the basic biological aspects of breast cancer. As we have elucidated, breast cancer carries complex genetic, epigenetic, and environmental factors in how it manifests in the individual patient. While the most common inherited genetic factor, the *BRCA1* and *BRCA2* gene mutations, have rightfully been studied in depth, upwards of 85% of breast cancers occur in women without apparent family history of the disease which includes the inherited *BRCA1/2* mutations. We hope to draw attention to the suggestion that naturally arises from this statistic that these cancers may be caused by genetic mutations that occur as a result of the aging process and lifestyle-related risk factors, rather than inherited mutations. We have reviewed how, while most breast cancers are carcinomas, common breast cancers can be divided into three major groups: non-invasive (or in situ), invasive, and metastatic breast cancers. Gene expression studies further identified several distinct molecular subtypes that differ significantly in prognosis as well as in the therapeutic targets present in the cancer cells. A continued specification of subtype identification is imperative in the development of individualized treatment. While it remains in its somewhat controversial nascence, the mammary stem cell theory may serve to shed light on the cellular origins of breast cancer. Normal breast development and mammary stem cells are regulated by some of the same signaling pathways including estrogen receptors (ERs), HER2, and Wnt/ $\beta$ -catenin signaling pathways, which control stem cell proliferation, cell death, cell differentiation, and cell motility. The heterogeneity and plasticity within breast cancers poses one of the most pressing issues for treatment. The recent evidence supports that epigenetic regulations and noncoding RNAs may play important roles in breast cancer development and may contribute to the heterogeneity and metastatic aspects of breast cancer, especially that of triple-negative breast cancer. Continuing progress in genomic and systems biology technologies will aid in understanding of the molecular and genetic bases of tumor heterogeneity and breast cancer metastasis, and will enable us to identify more reliable diagnostic and prognostic biomarkers for metastatic and recurrent breast cancers. We hope to encourage the further development of novel diagnostic and therapeutic measures to fully realize the best possible patient outcomes for those who suffer from breast cancer.

## Conflicts of interest

The authors declare no conflicts of interest.

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