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An overview of breast cancer: Classification and related signaling pathways

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Abstract: The burden of cancer continues to grow in developed and developing countries, with about 70% of all cancer mortality in low- and middle-income countries. Among different cancer types, breast cancer is recognized as one of the five most common causes of death in cancer in women worldwide, right after lung cancer. Histological classification divides breast tumors into different categories based on their behavior and clinical outcome. However, the histological classification system has some limitations, thus molecular subtype classification has been studied extensively to improve the classification system for breast cancer. Like any other cancers, several signaling pathways that enhance the proliferation, survival, invasion, and metastasis capability of tumor cells have been observed in breast cancer. These crucial signaling pathways contributing to the etiology of breast cancer include breast tumor kinase (BRK) pathway, Notch signaling, Nuclear Factor-kappaB (NF-κB) pathway, and human epidermal growth factor receptor (HER) pathway. In the present review article, we summarize our current understanding of breast cancer and its signaling pathways, which serve as basic information on tumor formation, maintenance, and expansion that could help form better breast cancer management in patients.

Keywords: breast cancer, signaling pathway, BRK, NF-κB, HER

1. Introduction

Cancer has become one of the top causes of morbidity and mortality, with approximately 18.1 million new cases and about 9.6 million deaths in 2018 based on the Global Cancer Observatory[1]. In other words, cancer is responsible for nearly one of the six deaths, leading to its recognition as one of the world's most prominent "killers"[1,2]. In addition to that, it has been suggested that nearly 65% of the increase in deaths related to all cancer types will occur in less developed regions of the world by the year 2030 while developing countries are anticipated to face an estimated burden of 23.6 million new cases per year[3–6].

As the leading malignancy in females, breast cancer cases continue to increase over the past few years, with a distinctive age-specific pattern reported by Bray and the team[1,7] By the same token, the disease may have an early onset age, occurring in younger women below 40 years of age and statistics showed a sharp increase in incidence rate right before menopause[1,8]. A recent report by Sharma, which extracted information from the GLOBOCAN 2018, stated that breast cancer had claimed an estimation of 626679 lives at an age-standardized rate of 13/100000[9]. In the same report, the highest incidence of breast cancer was noted in East Asia with 476509 cases (range: 474656–478370 cases), but the highest death counts due to breast cancer were observed South-central Asia at 123,060 deaths (range:119,256–126,986) in 2018. In Malaysia, the Health Facts 2013[10] released by the Ministry of Health (MoH) Malaysia highlighted cancer as one of the top ten causes of hospitalization and one of the top five causes of mortality (approximately 30000 cases annually). Based on the report released by the Malaysian National Cancer Registry Report (2016)[11], 103507 new cancer cases were diagnosed between 2007 and 2011 in Malaysia, and breast cancer appeared to be the most common cancer among female residents (32.1%) in Malaysia.

Along with the advancement of detection technology, the survival rate of breast cancer patient seems to have improved through surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted therapy[12]. While chemotherapy remained the broadest approach and first choice applied in inhibiting cancer cells' proliferation, it is often militated by numerous factors comprised of chemo-resistance, non-selectivity actions of chemotherapeutic agents, and high proliferation rates in breast cancer cells[13]. In fact, these prominent characteristics of cancer cells are often the result of abnormal activation of signaling pathways that granted these cells increased proliferation ability, along with invasion and metastasis capability. Therefore, a better understanding of underlying signaling pathways is essential, particularly in ensuring an excellent therapeutic plan and achieving the best clinical outcome.

2. Histological Classification of Breast Cancer Subtypes

The breast is comprised of two main types of tissue: (a) glandular tissues that house lobules (i.e., milk-producing glands) and ducts, and (b) stromal tissues, which provide the "supporting framework" of the breast (i.e., fatty and connective tissues) (Figure 1)[14]. However, due to the lack of biomarkers, the definition of breast cancer advancement remains challenging compared to other cancer types like colon cancer[15,16]. Through histological investigations, breast cancer can be differentiated based on its site and invasiveness. The invasive form of breast cancer is seen to breach the duct and lobular wall, invading the breast's supporting tissues. Despite that, this does not imply its metastatic capability, as some breast cancer can be invasive but do not spread further to other organs or lymph nodes. The other form of breast cancer is carcinoma in situ or non-invasive breast cancer, which can be subcategorized into two types: either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS). Generally, DCIS is considered as pre-invasive or non-invasive breast cancer and constitutes one in five new breast cancer cases reported. However, untreated DCIS may spread

over time by invading into adjacent breast tissue and progress into invasive cancer. The detection of DCIS has increased significantly following the use of mammography screening. In the United States, 90% of DCIS cases detected through mammography are observed as suspicious calcifications. DCIS is further categorized into several subtypes, primarily based on the morphological appearances: Comedo, Cribiform, Micropapillary, Papillary and Solid[17]. On the contrary, LCSI is rather a rare condition in which abnormal cells develop in the lobules at the terminal end of the duct. It is also displaying a worrying trend, given that the number of LCSI cases has multiplied over the past few years, with the highest rate observed among women aged between 40 to 50 years[18].

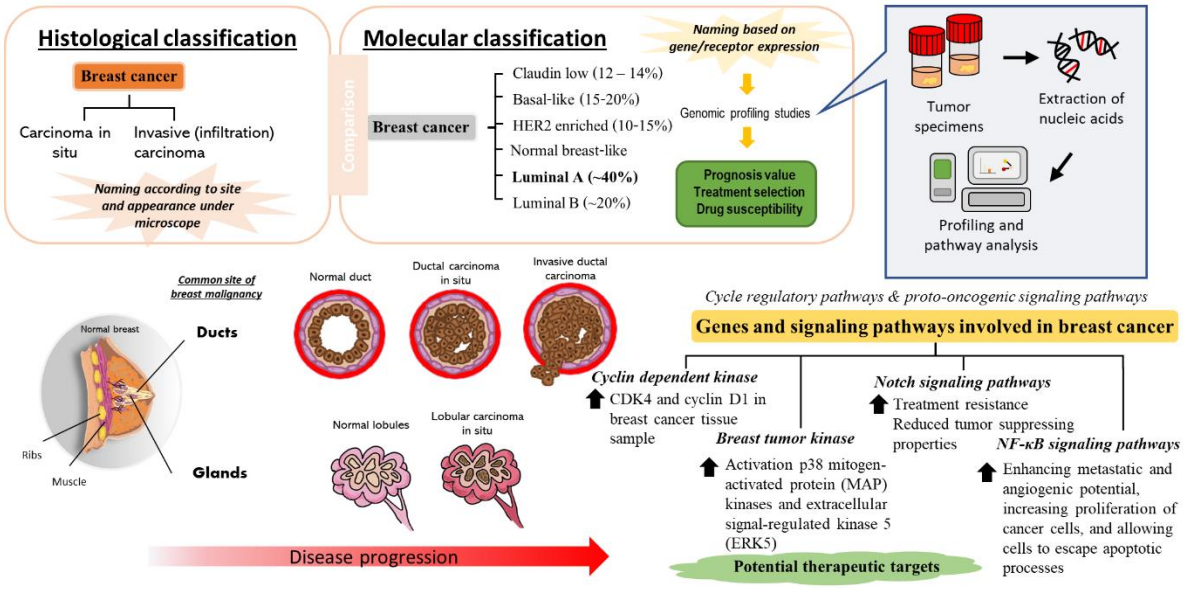

Figure 1. Classification of breast cancer subtypes and examples of signaling pathways involved in the etiology of breast cancer[19,20].

On the other hand, the major invasive tumor types are infiltrating ductal, invasive lobular, ductal/lobular, mucinous (colloid), tubular, medullary, and papillary carcinomas. Being the most prevalent type of invasive breast cancer, infiltrating ductal carcinoma (IDC) accounts for approximately 80% of all invasive breast cancers. As indicated by its naming, IDC arises in the breast's ductal region, invading through the duct wall, and subsequently expands into the supporting tissue of the breast. There are three grades of IDC: well-differentiated (grade 1), moderately differentiated (grade 2), or poorly differentiated (grade 3). The grading of IDC is carried on based on the rate of nuclear pleomorphism, glandular/tubule formation, and mitotic index, which aids in its prognosis[21].

3. Molecular Classification of Breast Cancer Subtypes

Originally assumed as a single disease, more evidence is emerging on breast cancer's complexity, suggesting it to be a group of diseases expressing distinguishable anatomical characteristics, reactive towards treatment and survival result. Current histological classifications greatly limit the ability to completely identify the clinical results of this

disease[22]. With the availability of more affordable molecular techniques, including genome sequencing technologies, researchers are now getting more insights into breast cancer subtyping via gene expression and genomic profiling studies. While the current model for breast cancer classification may offer some levels of prognostic value, the molecular classification of breast cancer offers more tremendous advantages in predicting the clinical outcome and, more importantly, assessing potential (distinctive) responses to specific therapies that assist clinicians in choosing the best therapeutic options for breast cancer patients[23,24].

Perou and team studied the gene expression of different breast cancer types using cDNA microarray profiling and sorted them into few intrinsic gene subtypes: basal-like breast cancer (BLBC) (estrogen receptor (ER) negative, progesterone-receptor (PR) negative, and human epidermal growth factor receptor-2 (HER2) negative), HER2-enriched, normal breast-like, luminal subtype A (ER+ and low grade) and luminal subtype B (ER+ and often high grade)[25–27] and the poorly described triple-negative subtype or "claudin-low" subtype [25–27] (Figure 1). Each of these molecular subgroups has a distinctive prognosis and chemotherapy sensitivity.

Additionally, the growth of breast cancer cells can be induced by hormones, estrogen, and progesterone. Thus, selecting specific therapy for breast cancer varies depending on these hormone receptors' expression[28,29]. Generally, 70% of breast cancer patients express ER and receive hormonal therapy treatment[28,30,31]. Studies also indicated that those with luminal-like cancers seem to have better long-term survival compared with other cancers, but those with basal-like and HER-2-positive tumors show higher susceptibility and sensitivity towards chemotherapy[26,32]. In contrast, patients diagnosed with triple-negative cancer have exceptionally shorter survival in first metastatic occurrence than other breast cancer types[33] .

By molecular classification, ER-positive luminal breast cancer is the most common type of invasive breast cancer. The ER-positive disease can be further classified into subtypes with different outcomes (i.e., luminal A and luminal B) using gene expression profiling with microarrays[34]. Luminal A tumors tend to have high expression of ER-activated genes, low-grade histological appearance, and slower growth, resulting in a better prognosis than luminal B tumors[35]. Furthermore, luminal A tumors are associated with the expression of luminal epithelial cytokeratins (CK) 8 and 18, other genes such as *hepatocyte nuclear factor 3 alpha (FOXA1), B cell lymphoma 2 (BCL2), erbB3, and erbB4*[36]. In comparison, luminal B tumors typically display a high proliferate rate and tumor aggressiveness, which is subsequently explained by its high relapse rate and lower survival rates than luminal-A breast cancer[37–40]. The main difference between these two luminal subtypes is the greater level of proliferation-related genes expressions such as *avian myeloblastosis viral oncogene homolog (v-MYB), gamma glutamyl hydrolase (GGH), lysosome-associated transmembrane protein 4-beta (LAPTMB4), nuclease sensitive element binding protein 1 (NSEP1)* and *cyclin E1 (CCNE1)* in *luminal-B* tumors[41]. It should be noted that luminal tumors form a continuum, thus the determination of these tumors into two subtypes based on proliferation may be arbitrary[42].

The HER2 is overexpressed in 15–30% of invasive breast cancer, mainly due to the overexpression of HER2/HER2 signaling-related genes and genes located in the HER2 amplicon on chromosome 17q12[25]. Even though HER2-enriched breast cancer can proliferate faster than luminal cancers, they are often highly responsive to targeted therapies aimed at the HER2 protein, resulting in remarkably improved outcomes[43]. The normal cell-like subgroup, comprising 5–10% of breast cancer patients[44]. Some researchers defined that this subtype was featured by similar gene expression to normal breast epithelium. Its proliferation rate is often low and responds to adjuvant chemotherapy[44,45].

The BLBC is an aggressive subtype that mainly occurs in young women and displays exceptionally high metastasis rates to the brain and lung[46,47]. This subtype is characterized by high histological grade, poor tubule formation, central necrotic zones, pushing borders, high mitotic indices, and proliferation rates[47]. Furthermore, the BLCL does not benefit from anti-estrogen hormonal treatment or trastuzumab as estrogen receptor (ER), progesterone receptor (PR), and HER2 were not expressed in this subtype. Therefore, a better understanding of the molecular background of BLBC to develop promising therapeutic regimes is necessary[48]. For instance, a study conducted by Hallett *et al*.[49] distinguished BLBC into two subgroups based on 14-gene. They assumed that this categorization might provide aggressive therapeutic regimes to the poor prognosis subgroup while avoiding such treatment in low-risk patients[50].

4. Genetic and Hormonal Risk Factors in Breast Cancer

In general, cell DNA impairment can cause mutations or chromosome rearrangements, which leads to carcinogenesis. Undoubtedly, breast cancer is a complex and multifactorial disease due to hormonal or genetic factors, or even a combination of both factors, as seen in many cases. Hormonal factors, main estrogen, play a vital role in breast cancer development[51]. Researchers hypothesized that excessive production of estrogen could stimulate the aberrant growth and development of organs affected by hormonal levels[52]. By this theory, hyperplasia tissue may pose as a "preview" before neoplasia development. However, while this is true, breast cancer risk appears to be predictable by the exposure to estrogen[53].

BRCA1 (Breast Cancer gene one) and *BRCA2* (Breast Cancer gene two) are two of the most important breast cancer susceptibility genes[54]. The *BRCA* genes play a critical role in cell damage repair and induce cell death to those cells if the damage is beyond rescue[55]. BRCA mutation leads to abnormal breast tissue proliferation and increases breast cancer risk[55,56]. It has been estimated that 5–10% of breast cancers diagnosed in women are associated with hereditary susceptibility, attributed to mutations in autosomal dominant genes, such as *BRCA1* and *BRCA2*[20,57]. Aside from that, 15–20% of female breast cancers occurred due to family inheritance but without a plausible autosomal dominant genes type[58]. There have been more than 500 sequence variations were identified since the isolation of *BRCA1*. Despite most are frameshift mutations, several missense mutations are reported that may alter the specific function of a protein. Furthermore, splice donor or acceptor site mutations are commonly reported[59]. The mutation spectrum of *BCRA2* may not as established as that of *BRCA1*.

However, the mutations reported in *BRCA2* mainly happen in exons 10 and 11 and always include insertions or deletions; these unwanted changes then cause missense alterations and premature stop codon seen in truncated and completely nonfunctional protein[60].

5. Signaling Pathways Involved in Breast Cancer

Knowing the exact mechanisms involved in any disease, including breast cancer, is essential before deciding on appropriate treatments. Lately, newer approaches are designed to target cell cycle regulatory pathways, proto-oncogenic signaling pathways targeted agents such as Notch, Wnt, SHH (Sonic hedgehog), ER (estrogen receptor), PI3k/AKT/MTOR, and HER2 (human epidermal growth receptor 2)[61]. Besides that, researchers also investigate the potential of focusing on the breast tumor microenvironment as a therapeutic target in breast cancer[62].

5.1. Cyclin Dependent Kinase

There are three main key families of molecules involved in cell cycle regulation, namely cyclins, cyclin dependent kinase (CDKs), and cyclin dependent kinase inhibitors (CDKIs)[63]. Dysregulation of the interplay between cyclins and their related CDK partners contributes to one of the hallmark features of cancer, a sustained proliferation of tumor cells[64]. CDKs could be either overactive or CDK-inhibiting proteins that are not in function in most cancers[61]. A study suggests that remarkable overexpression of CDK4 and cyclin D1 occurred in breast tumors. Consequently, it has been postulated that CDK4 is dispensable for the development of the normal mammary gland and poses as a suitable therapeutic target, specifically promote breast cancer cells inhibition while sparing other healthy cells[65,66],

5.2. Breast Tumor Kinase

The overexpression of breast tumor kinase (BRK) has been implicated in several malignancies such as prostate, ovarian, colon, and metastatic melanoma[67–70]. BRK is a non-receptor tyrosine kinase that is overexpressed in 60% of human breast tumors. Nevertheless, it is not expressed in normal human mammary gland and benign tumors[71,72]. A high BRK expression has been shown in invasive carcinoma, but it is also significantly expressed in HER2 and HER4[73,74]. A previous study demonstrated that BRK contributes to upstream of p38 mitogen-activated protein (MAP) kinases and extracellular signal-regulated kinase 5 (ERK5) as well as downstream of epidermal growth factor receptor (ErbB) expression[75]. The overexpression and constitutive activation of BRK breast cancer cells subsequently induced elevated cell survival and anchorage-independent growth, respectively[76,77]. Similarly, constitutive BRK expression also induces the EGFR tyrosine kinase pathway and upregulates breast tumor cell migration via paxillin and Mitogen-activated protein kinase (MAPK) activation, and enhances cancer cell proliferation through phosphatidylinositol 3-kinase (PI3K) and Akt expression[13,76–78].

Even though the exact cellular roles of BRK in breast cancer have yet to be fully delineated, recent data strongly imply that the deficiency of BRK in breast tumor cells can

activate EGFR-regulated signaling molecules, preceding breast cancer cell proliferation, migration, and elevated MAPK activity[79,80]. Hence, further investigation on the BRK role in breast cancer is needed to achieve the optimum treatment outcome.

5.3. Notch Signaling

Notch signaling has been identified for more than two decades in developing the human mammary gland, governing a plethora of cellular activities such as stem cell maintenance, regulation of cell fate, differentiation, and proliferation, motility, and survival. Alterations of these processes are known to enhance human breast cancer progression[81]. While mammals have four Notch homologues, but solid tumors such as breast cancers may co-express some Notch homologues that grant them resistance to highly selective therapeutic agents. The oncogenic role of Notch has been shown in breast cancer tumorigenesis through cross-talk with some other signaling pathways, including estrogen, human epidermal growth factor receptor 2 (HER2), Ras, and Wnt signaling pathway[82]. For example, approximately 80% of breast malignancies treated with anti-estrogens develop treatment resistance, and it is believed to be due to the involvement of the Notch pathway[83]. Therefore, targeting in both signaling pathways concurrently may help to overcome or delay this undesired resistance.

Despite that, some Notch homologues may confer inhibitory action against cancer cells. As described by O' Neill *et al.*[84], pro-oncogenic effects of Notch-1 and Notch-4 are counteracted by Notch-2 in human breast cancer cells. Notch-1 expression was found to be increased in poorly differentiated breast tumors, whereas expression of Notch-2 was increased in well-differentiated breast tumors. Additionally, a study suggested that Notch-1 may exert tumor-promoting properties while Notch-2 may possess tumor-suppressing functions[85]. The interactions between different Notch homologues could mean different cancer treatment outcomes, so taking a deeper look into these homologs' role could essentially assist the development of a promising treatment plan.

5.4. Nuclear Factor-kappaB (NF-κB)

The nuclear factor-kappaB (NF-κB) superfamily comprises transcription factors that take on a crucial role in regulating processes such as angiogenesis, cell proliferation, cell invasion, cell migration, metastasis, and apoptosis[86–89]. In normal cells, the NF-κB signalling pathway is tightly regulated. It can only be activated upon divergent stimulation of epidermal growth factor (EGF), bacteria and lipopolysaccharides (LPS), chemical and physical stresses, inflammatory cytokines such as interleukin (IL)-1 α and β as well as tumor necrosis factor-α (TNF-α)[90]. Upon degradation of IκB by IκB kinase (IKK) phosphorylation, the activation of NF-κB signaling pathways commences with free p50 (NF-κB1) and p65 (RelA) subunit release into the cytoplasm before further activation of downstream pathways that lead to the physiological responses[91,92].

Dysregulated activation of NF-κB signaling pathways, which drives abnormal expression, has been observed in various types of human cancers[93,94], including enhancing

tumor's metastatic and angiogenic potential, increasing proliferation of cancer cells, and allowing cells to escape apoptotic processes[93,95]. In reality, various antiapoptotic factors comprise of the BCL2 family (such as Bcl-xL and Bcl-2), cellular inhibitors of apoptosis (cIAPs) and caspase-8/FADD (FAS-associated death domain)-like IL-1beta-converting enzyme (FLICE) inhibitory protein (c-FLIP) can be activated by NF-κB signaling pathways[91,95].

Increased NF-κB activity can lead to abnormal chemokines expression and elevates cell migratory activity[92]. In line with that, several matrix metalloproteinases (MMPs) identified at κB sites can enhance cell invasion of surrounding tissue[96]. Active NF-κB also regulates the expression of specific molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial leukocyte adhesion molecule-1 (ELAM-1). These molecules are vital players in cancer metastasis, allowing vessel wall penetration to transport the cancer cells to distant parts of the body[86,95]. Likewise, high expression of NF-κB protein stimulates extracellular matrix destruction by tumor cells, boosting the tumor's metastatic ability[97,98]. All in all, inhibiting aberrant activation of the NF-κB pathway illuminates potential therapeutic options in suppressing human cancer[99].

5.5. Human Epidermal Growth Factor Receptor (HER)

Falling within the tyrosine kinase receptors family, the human epidermal growth factor receptor (HER) consists of four subfamilies and is usually expressed in normal tissues[100]. Amongst the subfamilies of HER, only human epidermal growth factor receptor 2 (HER2) is correlated to various breast cancers[101]. HER2 is found to be amplified in 20-30% of invasive breast tumors; its overexpression is associated with cancer cell proliferation, cancer development, and cancer cell metastasis, thus resulting in poor prognosis and a low survival rate in breast cancer patient[29,102].

The FDA has approved therapeutic HER2-targeted drugs against metastatic breast cancer. These drugs include trastuzumab, lapatinib, and pertuzumab[103,104]. As reviewed by Vranic and the team recently, these anti-HER2 drugs can improve the outcome of patients with HER2-positive breast cancer when used alone or in combination with other conventional chemotherapeutics[105]. However, trastuzumab resistance was reported to be developed in some patients, although a portion of them may show substantially improved outcomes[102].

6. Conclusions

Breast cancer is the most common multifactorial disease occurred in women and brought the highest rate of death. A general model of breast cancer carcinogenesis postulates that a normal cell achieves several new capabilities, including genome instability, proliferate infinitely, resistance to cell-death signaling, angiogenesis, and escape from immune surveillance. These pathways may either work independently or inter-signaling to each other, contributing to multi-drug resistance (MDR), which is now a major challenge reported for breast cancer chemotherapy. A better understanding of breast cancer signaling pathways could

help improve tumor treatment and prevent recurrence and metastasis. Therefore, therapeutic targeting in a specific signaling pathway would allow tailored treatments with more appropriate and effective individual responses, resulting in more personalized and conservative breast cancer interventions.

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