We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,100
Open access books available

149,000
International authors and editors

185M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Molecular Mechanisms of Breast Cancer Metastasis

*Nazlıcan Yurekli, Elif Cansu Abay, Merve Tutar, Ecem Cabri,
Kubra Acikalin Coskun, Alev Kural and Yusuf Tutar*

Abstract

Breast cancer (BC) is one of the most frequently occurring diseases with high morbidity and mortality rates in the world today. BC cells live under stress with altered pathway signaling, chromosome and microsatellite instability, aneuploidy, hypoxia, low pH, and low nutrient conditions. In order to survive and reproduce in these stressful environments, BC cells rapidly undergo adaptive mutations, rearrange their chromosomes, and repress tumor suppressor genes while inducing oncogene activities that cause the natural selection of cancer cells and result in heterogeneous cancer cells in the tumor environment. Unfortunately, these genetic alterations result in aggressive BC cells that can not only proliferate aggressively but also migrate and invade the other tissues in the body to form secondary tumors. In this review, molecular mechanisms of metastasis of BC subtypes are discussed.

Keywords: breast cancer, metastasis, heterogeneity, luminal A and B, TNBC, HER2+

1. Introduction

The breast tissue is made up of lobes, adipose tissue, ligaments, cavities (sinuses), glands, and milk ducts. Breast cancer (BC), which develops as a result of excessive cell growth in the breast tissue, is one of the leading causes of mortality after heart and vascular disorders. Although male BC is uncommon, female BC is the most frequent cancer. BC, like all cancers, causes a multitude of DNA abnormalities in healthy cells. As a result, cells begin to multiply uncontrollably. Cancerous cells reproduce and replicate more than healthy cells, and they live much longer. A tumor is defined as an aggregation of cells that results in the creation of a mass [1]. This syndrome frequently occurs in BC as a result of the fast growth of transformed cells in the milk ducts or mammary glands in the breast tissues. Cancer cells that grow in these locations generate a mass known as tumors. BC tumors can be in non-cancerous benign or in cancerous malignant forms. Both forms have varying impacts on the body. Cell growth, which leads to a malignant tumor, is generally gradual in the beginning and does not cause symptoms [2]. Despite advancements in early diagnosis and treatment strategies, metastatic BC continues to be an incurable disease [1].

The spread of malignant BC cells to different human tissues and organs is referred to as BC metastasis. Angiogenesis, invasion, migration, extravasation,

and proliferation are a few of the multistep, intricate, and interconnected chains of events that contribute to the development of cancer. By inducing the growth of new blood arteries, tumor cells first break their interactions with surrounding cells and detach from the underlying tumor tissue which is called Epithelial to Mesenchymal Transition (EMT). A primary tumor is one that has formed at the initial location of the tumor. A variety of specific transcription factors, " Epithelial-to-Mesenchymal Transition (EMT) inducers," are at least partially responsible for the complex genetic changes required to achieve EMT-associated phenotypic changes. Snail, Slug, SIP-1, δEF1, E12/E47, and Twist are included in transcription factors that induce EMT. These factors act as transcriptional repressors of E-cadherin in various cell types [1]. In addition, it has been reported to promote EMT by affecting many genes such as matrix metalloproteinase 9 or SPARC in metastasis and cancer invasion. In several cancer models, activation of the transforming growth factor—(TGF) signaling pathway and subsequent upregulation of the EMT inducers Snail, Slug, Twist, and ZEB have been reported to result in EMT [2]. In addition, FOXC2 is a transcriptional factor that promotes EMT and metastasis in vivo. It has been reported that this factor is associated with basal-like cancers [3].

Ten percent of cancer-related deaths are caused by original tumors, but 90% of cancer deaths are caused by metastases, which are secondary cancers that have grown outside the primary tumors. After exiting the main tumor tissue, BC cells move into the extracellular matrix where they advance and either occupy nearby tissues or enter the circulatory system to migrate to distant tissues. Lymph and blood arteries carry them from the main tumor's development site to the metastatic areas. As a result, they survive and reproduce themselves [1, 2]. Because metastasis is a multistep and complex process that includes different steps, due to the heterogeneity of BC, the mechanism of metastasis may diverge from the genetic background of the BC cells. BC has a lot of morphological and molecular heterogeneity not just across tumors, but even within a single tumor. Gene expression profiling allows the identification and classification of major subgroups with varying clinical characteristics and therapeutic responses. The difference between subtypes is caused by three tumor markers: estrogen (ER), progesterone hormone receptors (PR), and human epidermal growth factor receptor 2 (HER2). High levels of hormone receptor expression are observed in luminal A tumors. In addition, the subtype has HER2-negative, ER and/or PR positive, and has low levels of proliferation-related genes. This subtype not only has a modest growth rate, but also a favorable prognosis [1]. BC can show differences in the expression of the hormonal receptors as the result of different genetic alterations and rearrangements within the cell. These differences result in tumor subtypes of BC that show different strategies to survive and invade. Different genomic backgrounds in BC result in genetic variation that shows different mechanisms in cancer progression, especially in metastasis. In this review, metastatic features of BC subtypes are discussed.

2. Mechanism of metastasis in breast cancer subtypes

BC is a very common cancer type that can arise either genetically or environmentally. It is divided into different subtypes according to its genetic overcomes (**Figure 1**). Each subtype has its specific genomic character. Therefore, their inner signaling pathways' roles in metastasis are quite distinct.
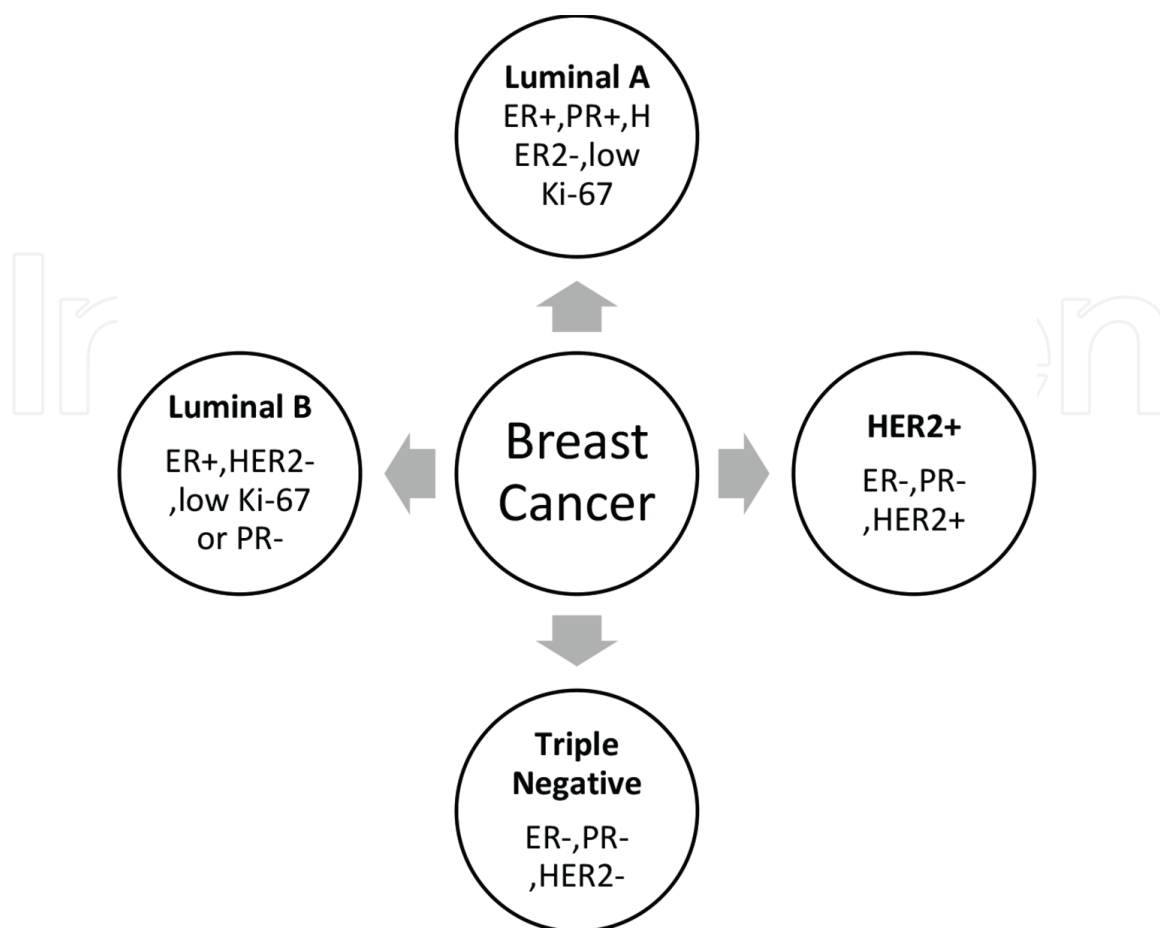
Figure 1.
Breast cancer is heterogenous cancer that includes 4 major subtypes according to their mutations. Estrogen receptors (ER) and progesterone (PR) receptors are specialized proteins found in certain cells in the body. Estrogen, progesterone, and female hormones circulating in the blood bind to these receptors and promote new cell growth and division. HER2 is a growth-promoting protein found outside of all breast cells (−, no expression; +, high expression).

2.1 Luminal A and B

ER-positive BC is divided into two types: luminal A and luminal B. It is shown to have different gene expression patterns, prognoses, and therapeutic responses. When compared to luminal A tumors, luminal B tumors have lower levels of ER or estrogen-regulated genes, lower or no expression of the PR higher tumor grade, higher expression of proliferation-related genes, and activation of growth factor receptor signaling pathways like IGF-1R and PI3K/AKT/mTOR [2]. Luminal B cancers, like luminal A tumors, are expected to have reduced endocrine sensitivity but have increased chemotherapy sensitivity [3].

Extravasation or lymphatic, local invasion, intravasation, colonization, and blood vessel migration are all steps in the tumor metastasis process. These processes then lead to metastases to distant organs [4]. The connection between tumor cells and the tumor microenvironment, which includes noncancerous cells such as immune cells, fibroblasts, adipocytes, and endothelial cells and as well as extracellular matrix (ECM), is crucial for organ-specific colonization [5]. The parallel progression model is more prevalent in breast tumors than the linear metastasis model. This suggests that BC cells spread early in the tumor's formation and that cancer cell spread may not be

dependent on tumor progression [6]. Several investigations have demonstrated that the genetic modifications of BC bone metastasis cells are not necessarily the same as those of their primary tumors. Distinct BC subtypes have been demonstrated to favor different metastatic sites, which are influenced by different molecular pathways. The molecular characteristics of BC and target organs appear to validate the organotropism of metastasis. All BC subtypes are prone to bone metastasis when compared to other subtypes; however, the luminal A subtype is an extraordinarily high-risk factor for bone metastasis. In addition, the prevalence of bone metastasis in luminal subtype malignancies is significantly higher (80.5%) than in HER2+ tumors (55.6%) or basal-like tumors (41.7%) [7]. Not only proliferation and metastatic capacities of BC subtypes are different, but also metabolic genotypes and phenotypes, vary with subtype. Nonetheless, metabolic alterations may differ not just within BC subtypes, but also depending on how tumor cells interact with their microenvironment [8]. This section highlights current knowledge about the association between metabolic programming, epigenetic modifications, and the metastatic process in BC. Understanding the metabolic processes that induce BC spread may lead to the development of new anticancer drugs.

Normal cells engage several signaling pathways in response to external growth signals and regulate glycolysis, oxidative phosphorylation (OXPHOS), and anabolic metabolism. Furthermore, unlike normal cells, which make adenosine triphosphate (ATP) largely by OXPHOS via the TCA cycle, most cancer cells rely on glycolysis for energy during aerobic conditions. The reverse Warburg effect, also known as metabolic coupling, is a metabolism that some tumor cells have. This mechanism not only results in chemotherapy resistance but also explains why some tumor cells have a high rate of mitochondrial respiration but a low rate of glycolysis [9, 10]. Moreover, the research identified a link between the luminal subtype and metabolically inactive reverse-Warburg/null phenotypes, whereas triple-negative breast cancer (TNBC) was linked to metabolically active Warburg/mixed phenotypes [11].

The expression of glucose transporter proteins (GLUTs) varies in BC and is connected to different clinical phases. In BC cells, GLUT1-5 and GLUT12 are active, although GLUT1 is the most important [12]. The pentose phosphate pathway (PPP) produces fructose-6-phosphate, nicotinamide adenine dinucleotide phosphate (NADPH), and ribose phosphate in addition to glycolysis and the TCA cycle [13]. Proteins involved in PPP are expressed in diverse ways in different molecular subtypes of BC. For instance, the HER2 subtype has greater expression of 6-phosphogluconolactonase and glucose-6-phosphate dehydrogenase than other BC subtypes, indicating a more active PPP [14]. Transketolase and G6PD expression have been associated with a worse overall and relapse-free survival rate in BC patients [15].

Glutathione and nicotinamide adenine dinucleotide (NADH) are the intermediates of glutamine and aid tumor cell proliferation and development by providing energy, supplementing glucose metabolism, and helping cells survive oxidative stress. Furthermore, certain tumor cells have developed an "addiction to glutamine", meaning that when there is no glutamine, they cannot survive [16]. Oncogenic transcription factors c-MYC and RAS can raise the metabolic activity of glutamine in tumor cells. At the same time, they can also upregulate some glutamine transporters including alanine-serine-cysteine transporter 2 (ASCT2) and enzymes involved in glutamine-to-glutamate conversion like glutaminase (GLS-1) [17]. Recent studies revealed that a greater glutamate-to-glutamine ratio particularly in ER-negative tumors was observed in breast tumor tissues. Glutaminase-1 (*GLS-1*), glutamate dehydrogenase (*GDH*), and *ASCT2* were found to be more strongly expressed in

HER2+ BC than in other subtypes. This indicates that HER2+ BC has the greatest glutamine metabolism activity. Importantly, the lowest expression of stromal *GLS1* and *GDH*, tumoral *ASCT2*, and serine hydroxymethyltransferase 1 were found in the luminal A subtype [18].

Amino acid biosynthesis and degradation, *de novo* nucleotide biosynthesis, reductive metabolism, and methylation are all involved in one-carbon metabolism. This metabolism has long been assumed to play a key role in sustaining tumor cells' high proliferation rate [19]. Additionally, folate (vitamin B9), and other B vitamins like B6 and B12, play an important role in one-carbon metabolism. Although the link between folic acid consumption and the risk of BC is still debated, a recent study found that increasing folate intake reduced the risk of ER-, ER-/PR- [20]. Immunity and tolerance are manipulated by tryptophan and arginine, which are frequently unregulated in malignancies. In BC contexts, the activity of arginase, the primary enzyme that catalyzes L-arginine, is increased, creating an adverse environment for T cell adaptability [21].

The development and progression of BC are dependent on lipid and fatty acids (FAs) metabolism [8]. By enhancing lipid and lipoprotein absorption or increasing cholesterol and lipid synthesis, cancer cells maintain a high rate of proliferation, displaying active lipid and cholesterol metabolisms [22]. Furthermore, the synthesis of FAs causes cancer cells to grow and proliferate faster. Fatty acid synthase (FASN) is a critical enzyme for FAs. When it is overexpressed, cancer proliferation occurs and a poor prognosis is observed in BC. That is why enhanced FAs activity is required for BC progression [23]. SREBP-1, a lipogenic transcription factor, can influence FASN expression by interacting with the *FASN* promoter region. *FASN* expression has also been shown to be influenced by the phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR and mitogen-activated protein kinase (MAPK) pathways [24]. The *FASN* expression is increased in BC cells because AKT and Sterol Regulatory Element-Binding Protein 1 (*SREBP-1*) are activated under hypoxic environments. Finally, inhibiting the MAPK pathway or using the mTOR inhibitor rapamycin can lower the expression of *FASN* in BC cells [25].

The signaling pathway of PI3K/AKT/mTOR is important for the cell cycle and metabolism in cancer development. Signals from growth factors, nutrients, energy signals, and various stress signals under hypoxia or DNA damage that provide growth and division of cells are integrated by the mammalian target of rapamycin complex 1 (mTORC1). The 110 genes in PI3K/AKT/mTOR pathway are often mutated in luminal BC. The most common *PI3K* mutations are found in around 40% of cases in luminal subtypes [26].

The alpha catalytic subunit of PI3K (PIK3CA) has been identified as the site of the bulk of *PI3K* mutations in ER-positive tumors. The most prevalent somatic mutation in BC is *PIK3CA*, which is seen in 36% of individuals with hormone receptor-positive, HER2-negative (HR+/HER2−) BC [27]. Crosstalk between the ER and the PI3K/AKT/mTOR signaling pathways has been proposed to be present during BC progression. Estrogens activate the PI3K/AKT/mTOR pathway, which allows ER cancers to migrate and invade distant tissues. mTOR signaling regulates the expression and activity of ER-α (one of two isoforms) in a reciprocal manner [8]. A recent study suggests that inhibition of the PI3K pathway activated the histone-lysine N-methyltransferase 2D (KMTD2), resulting in ER activation in BC cells [28]. Importantly, when AKT/mTOR signaling is activated by PI3K antagonists, the activation of energy-active mitochondria to the cortical cytoskeleton of cancer cells occurs. In this way, tumor cell invasion is increased. Inhibitors of the PI3K pathway slow cancer development, however, they

may promote tumor invasion by reprogramming mitochondrial transport, OXPHOS, and boosting cell motility [29].

ER-positive tumors exhibit lower levels of glycine, lactate, and glutamate (high glutamine), as well as reduced glutaminolysis. Therefore, this suggests that ER is involved in tumor metabolic control. Through interaction with many important regulators and pathways, including PI3K/AKT/mTOR, TP53, c-MYC, and Ras/Raf/ MAPK, ER plays a crucial role in metabolic control, allowing tumors to reprogram their metabolism to match diverse sorts of environments [29]. By activating ER-α, 17b-estradiol can increase insulin receptor expression while lowering the lipogenic activity of lipoprotein lipase in adipose tissue. Furthermore, Estradiol (E2) and ER-α can both control how the metabolism is reprogrammed in the presence of glucose. E2 promotes glycolysis by upregulating AKT kinase activity and inhibits TCA cycle activity in high glucose situations. On the other hand, in low glucose conditions, E2 activates the TCA cycle by upregulating PDH activity and suppresses glycolysis to meet the tumor cell's energy needs [30]. Importantly, recent research revealed that E2 appeared to promote glycolysis whereas tamoxifen inhibited it. E2 can upregulate *GLUT1* transcriptionally and so enhance glycolysis [31]. The other form of ER is ER-b. In high-grade BC, ER-b expression is downregulated or absent. ER-b, like ER-α, appears to boost glycolysis while suppressing OXPHOS in glucose metabolism. Multiple glycolysis-related pathways are elevated in ER-b-activated mammospheres, suggesting that ER-b plays a major role in regulating BC stem cell metabolism [32]. Epigenetic alterations are mostly enzymatic and possibly reversible. Methylation of DNA, acetylation of histone proteins, and changes in miRNA expression are all epigenetic alterations that affect protein synthesis patterns [33].

In mammalian cells, DNA methylation is one of the essential epigenetic changes. While it controls gene expression in normal development and growth, it is dysregulated in cancer. DNA methyltransferases (DNMTs) such as DNMT1, DNMT3a, and DNMT3b catalyze the methylation of CpG islands in DNA. DNMT1 is critical for methylation to be maintained during DNA replication in normal cells during mitosis. Its absence can result in hypomethylation. *De novo* methylation patterns are thought to be generated by DNMT3a and DNMT3b. DNMT1, DNMT3a, and DNMT3b expression levels are higher in BC than in normal breast tissue. When compared to DNMT1 and DNMT3a, the DNMT3b gene has the largest range of expression [34]. This suggests that DNTM3b is the primary actor in BC. Studies have shown that there are nearly 70% of methylated-CpG islands in the human genome and are found in closely packed core regions of DNA, where they affect gene silence and chromosomal integrity.

On contrary, unmethylated CpG islands are present in relaxed, the open state typically promoter regions of DNA. In this way, transcription factors and other regulatory proteins can access housekeeping and regulatory genes for expression. Normal cells are transcriptionally active. Because CpG islands that are present in the promoters of tumor-suppressor genes are frequently unmethylated in normal cells. On the other hand, in malignant tumors, hypermethylation of CpG islands that are found in promoters of tumor-suppressor genes is observed. Several studies have found and analyzed DNA methylation patterns and their association with breast cancer development and progression throughout the last decade. Cell cycle regulation (Ras Association Domain Family Member 1 -*RASSF1A*), Cyclin-Dependent Kinase Inhibitor 2A (*CDKN2A*), Cyclin-Dependent Kinase Inhibitor 1B (*CDKN1B*), Cyclin D2 (*CCND2*), DNA repair *(*BRCA1 DNA Repair Associated-*BRCA1*), MutL homolog 1 (*MLH1*), O-6-Methylguanine-DNA Methyltransferase (*MGMT*), cell

detoxification (glutathione S-transferase pi 1-*GSTP1*), apoptosis (Homeobox protein Hox-A5—*HOXA5*), the target of methylation-induced silencing (*TMS1*), cell adhesion and invasion (Twist-related protein—*TWIST*), Cadherin-1 (*CDH1*), metalloproteinase 3 (*TIMP3*), hormone receptors (*ESR1* and progesterone—*PGR*) are among the genes that methylated and thus are silenced [35]. The most important genes for breast cancer, *BRCA1*, and *BRCA2* are tumor suppressor genes that maintain genomic stability by participating in homologous recombination repair and gene conversion of double-stranded DNA breaks. Mutations in BRCA1 and BRCA2 tend to develop breast cancer. Loss of BRCA function due to pathogenic mutations in BRCA causes a lack of homologous recombination. *BRCA1* tumors are high-grade and negative for hormone receptors as well as have a high proliferation rate. Also, *BRCA1* tumors are positive for some cell cycle promoter genes. *BRCA2* tumors, on the other hand, present an opposite phenotype to *BRCA1* tumors but are very similar to sporadic tumors except for *BRCA2*. A research proposed that BRCA1 carriers may be more likely to develop triple-negative cancers and also develop invasive ductal carcinomas of high nuclear, histological grade, and hormone receptor-positive tumors are more common in *BRCA2* mutation carriers [35].

A total of 220 different DNA methylation sites in malignancies were examined. It is demonstrated that with these loci, normal and benign tissues of BC are distinct [36]. Genome-wide researches on breast tumors demonstrated that large number of genes have hypermethylation patterns, known as the "CpG island methylator" phenotype. This phenotype has some advantages [37]. For instance, it is protective, with a specific epigenomic profile linked to reduced metastatic risk and longevity. In contrast, a significant risk of metastatic disease and mortality is observed in the absence of this phenotype. In addition, DNA methylation patterns can be different in BC subtypes [38]. Luminal B tumors are more commonly methylated than basal-like or TNBC [39]. As a result, it is clear that methylation has a substantial role in distinct subgroups of BC and it will be crucial to elucidate the mechanisms in the methylation states. In this way, BC may be targeted therapeutically. Last, but not least, the DNA methylation pattern in endocrine-resistant cancer might give precise indicators to identify and predict the response to therapy. Thus, drugs that target particular enzymes that have crucial roles in epigenetic alterations are being developed and evaluated [38].

Ubiquitination, phosphorylation, and SUMOylation are all examples of post-translational modifications of histone tails. However, acetylation/deacetylation and methylation are well-studied modifications to the expression of genes. The acetyl groups from ε-amino groups of lysine residues are removed by histone deacetylases (HDACs). In this way, chromatins are compacted into well-ordered nucleosomes, preventing transcription factors from accessing DNA. Histone acetyltransferases (HATs) acetylate the lysines, loosening chromatin and facilitating transcription factor binding. When histones are methylated, the genes are generally turned off. On the other hand, when histones are demethylated, the genes are turned on by loosening histone tails. In a summary, histone methylations prevent DNA to be bound by transcription factors, therefore controlling the activity of genes. HDACs and HATs are divided into various groups, each of which catalyzes a different biological process [40].

Based on their structure, HDACs are divided into two groups: zinc-dependent class I, IIa, IIb, and IV, and zinc-independent class III. According to their chemical structure, HDAC inhibitors are classified into four classes: hydroxamic acids, cyclic peptides, short-chain FAs, and benzamides. Some of them can inhibit cancer cell proliferation and promote apoptosis by repressing silenced genes. Vorinostat and

other HDAC inhibitors including entinostat and panobinostat (LBH-589) are being studied in several Phase I and II clinical trials for the treatment of BC. Moreover, their use in combination with standard cytotoxic (paclitaxel) and endocrine (tamoxifen) therapies, as well as therapies targeting HER2 (Herceptin; trastuzumab) or Vascular endothelial growth factor (VEGF), (Avastin; bevacizumab). A combination therapy that uses HDAC inhibitors and DNMT inhibitors works together to re-express suppressed genes, causing apoptosis and reducing tumor metastasis [41].

Lysine (K) and arginine (R) residues restrict histone methylation, with lysins being the most prevalent. Lysine methyltransferases and demethylases reverse the process. Active transcription is linked to methylation of histone H3 lysine 4 (H3K4), H3K36, or H3K79, while gene silencing is linked to methylation of H3K9, H3K20, or H3K27 [42]. Enhancer of zeste homolog 2 (EZH2) is a highly conserved histone methyltransferase that acts as a transcriptional repressor and methylates H3K27. Overexpression of the *EZH2* is linked to aggressive and metastatic BC tumors. The EZH2 inhibitor 3-Deazaneplanocin (DZNep) promotes apoptosis in BC cells although this is not the case in normal ones. Tanshindiols are EZH2 inhibitors that also have an anticancer effect in a variety of tumor cell lines. Last but not least, inhibitory EZH2 peptides have been developed, one of which, SQ037, has been verified and found to have significant anti-EZH2 potency. These reagents show how specificity may be tailored to create medications that specifically target epigenomic enzymes and have the desired effect with minimum adverse effects [43].

The methyltransferase *SMYD3*, which is overexpressed in various tumors, including BCs, targets H3K4. The use of short interfering RNAs to silence *SMYD3* decreases the development of cancer cells. Novobiocin suppresses the proliferation and migration of MDA-MB-231 BC cells via inhibiting *SMYD3* expression. Tranylcypromine is another powerful H3K4 methylase. This tiny chemical demethylation inhibitor inhibits the transcription of key target genes, including the pluripotent stem cell marker *OCT4* [44]. LSD1 demethylates H3K4, as well as nonhistone proteins including p53 and DNMT1. This indicates that it has a wide range of biological roles. When histone-modifying enzymes like LSD1 and EZH2 are overexpressed, they silence essential genes like tumor suppressor genes. Inactivation of these proteins is suspected to have a role in the development of BC and other cancers. However, because LSD1 is abundantly expressed in ER-breast tumors and is a hallmark of aggressiveness, its control in malignancies needs further investigation [45].

In metastasis, miRNAs perform a unique role: while overexpression of a few miRNAs leads to metastasis, the expression of some miRNAs suppresses metastasis. Inflammation and BC metastasis suppressor 1 (*BRMS1*)-mediated metastasis suppression are both controlled by miR-146. Overexpression of miR-146a/b in MDA-MB-231 cells resulted in a substantial drop in epidermal growth factor receptor (*EGFR*) expression, as well as decreased migration, invasion, and metastasis to the lungs [46]. Additionally, in human BC cells, expression levels of miR-335 and miR-206 decreased as the metastatic potential increased. Although a decrease in the expression of these miRs in cancer cells reduced lung and bone metastases, the initial tumor size had no effect. miR-335 inhibits metastasis via regulating the expression of *SOX4* [47].

In BC cells, studies show a negative association between miR-142-3p and the migration of cells. When the miR-142-3p expression is suppressed, the expression of proteins such as zinc finger E-box binding homeobox 1 (*ZEB1*) and Ras-related C3 botulinum toxin substrate 1 (*RAC1*), that allow for the development of an invasive phenotype increases. Additionally, recent research has indicated that overexpression of miR-142-3p has been linked to the suppression of BACH-1, MMP9, chemokine

receptor CXCR4, and vascular endothelial growth factor receptor (VEGFR) protein expression in BC cells [48]. miR-17-5p has been shown to have a unique antimetastatic action in recent investigations. Suppressing miR-17-5p resulted in increased pro-metastatic gene expression and increased metastasis to the lungs. On the other hand, intratumoral delivery of miR-17-5p mimics decreased lung metastasis considerably. Moreover, reduced miR-1179 expression in BC was linked to advanced clinical stage and metastases to the lymph node, according to a clinicopathological study [49]. When miR-1179 is upregulated, it suppresses BC cell proliferation and metastasis by regulating the expression of *NOTCH1*, *NOTCH4*, and its downstream modulators, *HES1* [50]. Last but not least, miR-21 overexpression in MDA-MB-231 cells decreases tumor invasive and metastatic characteristics. On the contrary, reduced miR-21 expression enhanced these cells' migration and invasion capabilities [51].

2.2 HER2(+) breast cancer

Human Epidermal Growth Factor Receptor 2 (Her2) protein is a transmembrane receptor tyrosine kinase, which is a member of the EGFR family and plays an important role in mitogen signaling. Amplification of this receptor plays an important role in BC. Overexpression of the Her2 protein results from the Erb-B2 Receptor Tyrosine Kinase 2 (*ERBB2*) gene amplification of all BC tumors (known as HER2-positive BC) [52]. *Her2* overexpression is caused by overactivation of the downstream phosphatidylinositol 3-kinase/ protein kinase B (PI3K/Akt), *Phospholipase* C, gamma 1 *(PLC-γ)*, and mitogen-activated protein kinase (*Mapk*) pathways, leading to increased tumor cell growth, survival, motility, and invasion [53]. *HER2* amplification and/or overexpression causes its conversion from a protoncogene to an oncogene. This has important effects on the metastasis of BC. Clinical studies show that amplification of *HER2* has a significantly worse prognosis in BC patients compared to patients with unamplified *HER2* [54]. In addition to the presence of HER2 in the membrane, it is also found in the nucleus at a lower rate than in the membrane. Despite low HER2 in the nucleus, it is thought to have important roles in the nucleus and chromatin [55]. HER2 is transported to the nucleus by endocytosis via importin β1 and the nuclear pore protein (NUP358) [56]. When HER2 enters the nucleus, it joins forces with PR and Activator protein 1 (AP-1) to activate the transcription factor Signal transducer and activator of transcription 3 (STAT3) in a complex [57, 58]. HER2 interacts with RNA pol I and actin, which enhanced growth by increasing the transcription of the rRNA gene [59]. In another study, it was reported that HER2 binds to the Cyclooxygenase (COX) promoter, which is associated with several malignant tumors found in SK-BR-3 (Skbr3) and BT-474 BC cell lines [60]. In a sizable cohort of BC patients, Dillon et al. [61] showed a correlation between *COX-2* expression and *HER2,* and *HER2* predicted poor disease-free survival in patients receiving endocrine therapy.

Tumor cells may become more vulnerable to further genetic harm and develop extensive instability in the tumor genome as a result of early genetic alterations that de-regulate tumor suppressors and oncogenes. Another research reported that comparative genomic hybridization to measure global copy number alterations discovered that HER2-amplified tumors had considerably greater levels of aberrations than HER2-negative tumors, indicating that these cancers were genetically more progressed [62]. The positive correlation between chromosomal changes at chromosomes 11q13.1, 16q22-q24, and 18q21 and HER2 amplification suggests that genes in these regions may be involved in the pathogenesis of HER2+ tumors in addition to the high levels of overall genomic instability associated with *HER2* amplification [63].

Depending on the metastatic stage, cancer cells may employ one or several metabolic pathways [64]. In addition, depending on where they metastasis, cancer cells may adopt a particular metabolic pattern [65]. Cytoplasmic and mitochondrial nuclear crosstalk can regulate the metabolism of BC. Metabolites in the cytoplasm and mitochondria dictate gene transcription and DNA methylation. Numerous transcription factors shuttle between the nucleus and mitochondria to ensure that genes that regulate metabolism are transcribed [66]. *HER2*-mediated signals regulate lactate dehydrogenase-A levels, 6-Phosphofructo-2-kinase levels, and lactate accumulation in tumors because they promote glucose utilization [66–69]. It has been reported that *HER2* can be replaced by the heat shock protein associated with mitochondria (mtHSP70), both in patient samples and in many cell lines. *HER2* in mitochondria has a negative regulatory effect by indirectly promoting glycolysis of oxygen consumption [70]. In another study, higher levels of glycine, succinate, creatinine, and glutamine were observed in *HER2+* tumors compared to *HER2-* tumors, while a decrease in alanine levels was reported [71]. *HER2* promotes the RAS-ERK-RSK pathways, ensuring cell survival. HER2+ inhibitors of HER2 in the mammary gland cause a decrease in the activity of these pathways. This, in turn, inhibits the survival of the cell. Another way is the AKT-mTOR pathway. This pathway causes cell proliferation. The HER2 inhibitor causes a decrease in the activity of this pathway. The rapamycin and rapalogs inhibit the activity of mTOR, such as in HER2+ BC, and prevent the phosphorylation of S6K, the process inhibits cell proliferation and reduces aerobic glycolysis as the result of the downregulation of glycolytic enzymes. In addition, the glucose analog 2-deoxy-D-glucose (2-DG) inhibits mTOR signaling by activating PI3K signaling, suppressing aerobic glycolysis, and phosphorylating AMPK on T172 [72].

HER2 expression in epithelial-like BC cells is significantly higher than in mesenchymal-like BC cells. This is because of the open/active chromatin of the *ERBB2* gene in epithelial-like cells, as well as the closed/inactive chromatin of the *ERBB2* gene in mesenchymal-like BC cells. The chromatin-based epigenetic silencing of the *ERBB2* gene in the EMT of HER2+ BC cells causes inhibition of *HER2* expression, which in turn leads to the emergence of resistance to anti-*HER2* monoclonal antibodies such as trastuzumab [73]. In this study, the H3K9ac and H3K27me3 epigenetic profiles and microanalysis of genes enriched with the promoter h3k9ac chip revealed epi-promoter regions of genes modified by mark at HER2+ and TNBC tumors. The H3K9ac modification has been reported to induce downregulation of most of the related genes in *HER2*-amplified tumors [74]. Descriptive investigations of the HER2/neu epigenetics in BC support the repression of *Her2/neu* by increased H3K9me2. Lim et al. demonstrated that the histone demethylase Kdm1, which removes the methyl groups from dimethylated H3K9, directly targets *Her2/neu*. In this instance, siRNA mediates *Kdm1* knockdown and reduces *Kdm1* accumulation on the *Her2/neu* promoter, which increases H3K9 methylation, decreases *Her2/neu* expression, and inhibits the proliferation of the treated BC cell lines [75]. In another study, it was determined that LAQ824 treatment caused the activation of Her2/neu transcriptional repressor, and acetylation of HSP90, on the other hand, it caused phosphorylated mitogen-activated protein kinase levels and hyperacetylation of HSP 90 with a labile chaperone complex. LAQ824 indirectly marked the Her2/neu protein for proteasomal degradation [45]. For several primary cancers, endocrine organs are metastatic targets. Primary tumors can spread directly or metastasize through the lymphatic and arterial routes. Melanomas, breast, and lung carcinomas are the primary tumors that metastasize to the adrenals most frequently. These tumors can cause adrenal insufficiency, especially

when both adrenals are affected. The most typical primary malignancies that metastasize to the pituitary are breast and lung tumors, which cause pituitary dysfunction in around 30% of cases [76].

Further, cyclin-dependent kinase pathways of PI3K/AKT may lead to endocrine resistance in treatments. Estrogen activity at the molecular level can induce activation of the PI3K/AKT and MAPK pathways at the cell surface and decrease ER and PgR expression [77]. Upregulation of the PI3K/AKT/mTOR pathway contributes to anti-estrogen resistance by promoting survival, tumor cell growth, motility, and metabolism. In this case, the ER promotes transcriptional activity [78]. The intrinsic properties of tumor cells, both soluble factors and ECM proteins from the microenvironment influence the response to Her2-targeted lapatinib or neratinib (TKI). In a study of growing cells on microenvironment microarrays (MEMA), both soluble and ECM factors from various microenvironments were reported to reduce responses to Her2-targeted TKIs. In addition, resistance-conferring factors differed between luminal-like (L-*Her2+*) and basal-like (*Her2E*) *Her2+* subtypes as defined (Cancer Genome Atlas Network, 2012). Microenvironment-mediated resistance was reversed when pertuzumab-treated L-*Her2+* cells co-treated with crizotinib in *HER2E* cells. Hepatocyte growth factor and neuregulin1–1 conferred resistance in *HER2E cells*, but not vice versa, in L-*Her2+* subtype cells. These varied responses to microenvironmental variables are the result of basic variations in the design and wiring of the signaling networks between the two subtypes. In *L-HER2+* cells and *HER2E* cells, co-treatment with crizotinib and pertuzumab successfully restored the microenvironment-mediated resistance. The findings in this study were consistent with studies that showed that *HER2E* and *L-HER2+* represent different diseases. The results suggest that *Her2+* subtype-specific approaches to block resistive microenvironmental signals may enhance clinical management of *Her2+* BC with *Her2*-targeted TKIs lapatinib and neratinib [79].

2.3 TNBC (HER-, ER-, PR-)

TNBC forms 10–15% of all BCs [80]. The cells from this subtype test as negative for the receptors of estrogen and progesterone hormones and also for HER2 protein [80]. When compared to other types that are hormone receptor-positive and HER2+, TNBC is generally more aggressive while being difficult to treat with its insufficient treatment options since hormonal therapy medicines or medicines that target HER2 protein is not available for this situation [81]. TNBC also shows to have a worse prognosis due to the development of metastasis in secondary organs like the brain, lungs, and bone [82]. The complexity in the metastatic process when combined with the lack of targeted therapy makes this disease a harder one to cure. Besides these problems, it is also found to be more likely to reoccur. But its symptoms, staging, diagnosis, and survival are similar to other invasive ductal carcinomas [83].

The basal-like subtype of BC is characterized by high proliferation, high histological grade, and poor prognosis. And this subtype can be triple negative although not all of the basal-like cancers resemble the forms that express ER and HER2 [82]. By gene expression profiling, TNBC is also identified with seven subtypes: two basal-like (BL1 and BL2), a mesenchymal (M), a mesenchymal-stem cell-like, an immunomodulatory, a luminal androgen receptor/luminal-like, and an unclassified type. And each subtype shows unique ontologies and different responses to standard-of-care chemotherapy [84]. Besides the differences, TNBC is generally found to be responding less to conventional chemotherapy while the patients carry a bigger risk of recurrence and relapse [85].

Non-coding RNAs (ncRNAs) may have a role in the progression of BC cases and the metastasis process. And in the process of forming miRNAs, Heterogeneous nuclear ribonucleoproteins A2/B1 (*HNRNPA2B1*) interacting with a component of the DROSHA complex is known to stimulate the processing of pri-miRNA to pre-miRNAs [86]. Also, *HNRNPA2B1* transcript and protein expression were found to be high in BC cells and tumors when compared to nontransformed cell lines and normal breast tissue. With the TNBC subtype, it is shown that MDA-MB-231 TNBC cells with *HNRNPA2B1* knockout have reduced tumor growth but were stimulated in metastasis when injected into mice. So, the role is not clear with *HNRNPA2B1* but it is essential to research deeply to understand its role in BC metastasis. Also, using the sublines of MDA-MB-468 TNBC cells, drivers of metastasis are identified as IL11 and VEGF-D in *in vivo*. They activate the effector neutrophils and promote metastatic niche [87]. In this case, chemotherapy may have a negative effect by increasing the metastatic potential if the cells with innate resistance are selected.

MetastamiRs are the miRNAs have a pro- or anti-metastatic effect [88]. Pro-metastatic miRNAs are expressed higher in breast tumors showing a link to reduced disease-free survival (DFS) and survival by miR-9-5p is one of them as a pro-meta-static oncomiR and found to be at a higher level in TNBC than other subtypes. miR-373, miR-29a/b/c, and miR-19a are all found to be pro-metastatic and with a high level in TNBC subtypes. miR-206, and miR-31-5p are listed as anti-metastatic with a lower expression in TNBC subtypes. But miR-20a-5p which also is listed as anti-metastatic has a higher expression in TNBC compared to other types [87]. All of the miRNAs affect the regulation of the metastasis process. The increased expression of miR-520c-3p is observed in MDA-MB-231 TNBC cells. And it is found to be inhibiting TGF-β signaling which can be related to inhibiting phosphorylation of suppressors against decapentaplegic 2 (SMAD2) and decapentaplegic 3 (SMAD3) and decreases target genes *ANGPTL3*, *PTHLH*, and *SERPINE1* (*PAI-1*) [89]. miR-373 is another miRNA that is pro-metastatic and with its increased expression, MDA-MB-435 cell migration and invasion are induced in *in vitro*. It has also been found to be promoting tumor metastasis observed via tail vein injection mouse model [87]. Some genes are found to be linked with TNBC metastasis. In a study, 26 hub genes were identified as metastasis-associated candidate genes [90]. In-depth studies with four of them, Immunoglobulin Superfamily Member 10 (*IGSF10*), Runt-related transcription factor 1 translocation partner 1 (*RUNX1T1*), X-inactive specific transcript (*XIST*), and transcription factor teeshirt zinc finger homeobox 2 (*TSHZ2*) indicated that they were downregulated in TNBC tissues and these genes have prognostic and diagnosis values in TNBC [90]. *IGSF10* is an immunoglobulin superfamily member 10 normally associated with developmental processes and differentiation [91]. By whole-exome sequencing it was found to be a potential cancer-related gene *RUNX1T1* is a member of the mind the gap (*MTG*) family. It was already known to be reported in many cancer types as a novel biomarker or as being vital for tumorigenesis. *XIST* plays a role in the inactivation of the X chromosome. It was also known to have a relationship with cancer cases since its expression was observed to be dysregulated in some cases. *TSHZ2* is a member of the TSHZ family and like the others, its expression was observed in other cancer types and was found to have a downregulation in some cancers [90].

With the studies done in TNBC, it is found that this subtype is possess more therapy-resistant Cancer Stem Cells (CSC) when compared to other subtypes. The difference in mortality and recurrence rates with the therapy failures may be the result of this difference in CSC enrichment in TNBC. So, studying cellular signaling

pathways and transcription factors that contribute to stemness can show an insight into this subtype. Notch signaling is a developmental pathway triggered by Notch ligands binding to Notch receptors. Its expression in BC CSCs promotes self-renewal and metastasis. TNBC is significantly deregulated compared to the other BC subtypes. Also, it is found that hypoxia which is a hallmark of TNBC can induce Jagged1, a Notch ligand, expression in TNBC CSCs and lead to metastasis and self-renewal. PKD3 is part of the protein kinase D (PKD) family and is shown to have a role in increasing TNBC metastasis, proliferation, and stemness. Another pathway that is linked to the increased risk of metastasis in TNBC is the Interleukin/Janus kinase-2/ signal transducer and activators of transcription-3 (IL-6/JAK2/STAT3) pathway and it is preferentially activated in TNBC CSCs comparing to the other BC subtypes. SRY-box transcription factor 2 (*SOX2*) is a stem cell pluripotency regulator which is effective in embryonic development and it is over-expressed in TNBC. This expression level of *SOX2* is associated with increased proliferation and metastasis. Further, Lipase H (*LIPH*) is found to regulate *SOX2* so it is another gene to promote metastasis in TNBC CSCs. *NANOG* is known to be a self-renewal and pluripotency regulator and it is also found to be a key driver of metastasis too. Ubiquitin carboxyl-terminal hydrolase 1 (*USP1*) and Protocadherin-7 (*PCDH7*) were also found to be promoting metastasis in TNBC CSCs. And finally, under the list of epigenetic regulation, histone methyltransferase *EZH2* was found to maintain metastasis in TNBC CSCs [85].

3. Future perspective

BC signaling pathway is complex and in certain immune subtypes and at different stages, the pathways may cross-talk. CSCs enhance the cellular environment and the heterogeneity burden of anticancer treatment. Therefore, elucidating molecular mechanisms is complicated. Further, clinical drugs may cause resistance, and patients may not give a similar response to cancer treatment. However, computer-based algorithms and designing similar compound patterns with modified side chains smooth drug design studies. Omics technologies highlight molecular correlations and coupling the knowledge with drug design provides innovative solutions. Non-coding elements also help our understanding of the molecular mechanism. Altogether, new perspectives in anti-cancer treatment may provide comprehensive and contemporary solutions.

Author details

Nazlıcan Yurekli[1], Elif Cansu Abay[1], Merve Tutar[2], Ecem Cabri[3],
Kubra Acikalin Coskun[4], Alev Kural[5] and Yusuf Tutar[6,7,8]*

1 Hamidiye Faculty of Medicine, Division of Medicinal Biology, Department of Basic Sciences, University of Health Sciences, İstanbul, Turkey

2 Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey

3 Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, Turkey

4 Faculty of Medicine-İstanbul Aydin University, Division of Medicinal Biology, Department of Basic Sciences, İstanbul, Turkey

5 Hamidiye Faculty of Medicine, Division of Medicinal Biochemistry, Department of Basic Sciences, University of Health Sciences, İstanbul, Turkey

6 Hamidiye Faculty of Pharmacy, Division of Biochemistry, Department of Basic Pharmaceutical Sciences, University of Health Sciences-Turkey, Istanbul, Turkey

7 Division of Molecular Oncology, Hamidiye Health Sciences Institutes, University of Health Sciences-Turkey, Istanbul, Turkey

8 Validebağ Research Center, University of Health Sciences-Turkey, Istanbul, Turkey

*Address all correspondence to: ytutar@outlook.com

IntechOpen

References

[1] Tabor S, Szostakowska-Rodzos M, Fabisiewicz A, Grzybowska EA. How to predict metastasis in luminal breast cancer? Current solutions and future prospects. International Journal of Molecular Sciences. 2020;**21**(21):8415. DOI: 10.3390/ijms21218415

[2] Sotiriou C, Neo SY, McShane LM, Korn EL, Long PM, Jazaeri A, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(18):10393-10398. DOI: 10.1073/pnas.1732912100

[3] Rouzier R, Perou CM, Symmans WF, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clinical Cancer Research. 2005;**11**(16):5678-5685. DOI: 10.1158/1078-0432.CCR-04-2421

[4] Pelon F et al. Cancer-associated fibroblast heterogeneity in axillary lymph nodes drives metastases in breast cancer through complementary mechanisms. Nature Communications. 2020;**11**(1):404. DOI: 10.1038/s41467-019-14134-w

[5] Ungefroren H, Sebens S, Seidl D, Lehnert H, Hass R. Interaction of tumor cells with the microenvironment. Cell Communication and Signaling: CCS. 2011;**9**:18. DOI: 10.1186/1478-811X-9-18

[6] Klein CA. Parallel progression of primary tumours and metastases. Nature Reviews. Cancer. 2009;**9**(4):302-312. DOI: 10.1038/nrc2627

[7] Smid M, Wang Y, Zhang Y, Sieuwerts AM, Yu J, Klijn JG, et al. Subtypes of breast cancer show preferential site of relapse. Cancer Research. 2008;**68**(9):3108-3114. DOI: 10.1158/0008-5472.CAN-07-5644

[8] Wang L, Zhang S, Wang X. The metabolic mechanisms of breast cancer metastasis. Frontiers in Oncology. 2021;**10**:602416. DOI: 10.3389/fonc.2020.602416

[9] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science (New York, N.Y.). 2009;**324**(5930):1029-1033. DOI: 10.1126/science.1160809

[10] Fu Y, Liu S, Yin S, Niu W, Xiong W, Tan M, et al. The reverse Warburg effect is likely to be an Achilles' heel of cancer that can be exploited for cancer therapy. Oncotarget. 2017;**8**(34):57813-57825. DOI: 10.18632/oncotarget.18175

[11] Choi J, Kim DH, Jung WH, Koo JS. Metabolic interaction between cancer cells and stromal cells according to breast cancer molecular subtype. Breast Cancer Research: BCR. 2013;**15**(5):R78. DOI: 10.1186/bcr3472

[12] Young CD, Lewis AS, Rudolph MC, Ruehle MD, Jackman MR, Yun UJ, et al. Modulation of glucose transporter 1 (GLUT1) expression levels alters mouse mammary tumor cell growth in vitro and in vivo. PLoS One. 2011;**6**(8):e23205. DOI: 10.1371/journal.pone.0023205

[13] Patra KC, Hay N. The pentose phosphate pathway and cancer. Trends in Biochemical Sciences. 2014;**39**(8):347-354. DOI: 10.1016/j.tibs.2014.06.005

[14] Choi J, Kim ES, Koo JS. Expression of pentose phosphate pathway-related

proteins in breast cancer. Disease Markers. 2018;**2018**:9369358. DOI: 10.1155/2018/9369358

[15] Benito A, Polat IH, Noé V, Ciudad CJ, Marin S, Cascante M. Glucose-6-phosphate dehydrogenase and transketolase modulate breast cancer cell metabolic reprogramming and correlate with poor patient outcome. Oncotarget. 2017;**8**(63):106693-106706. DOI: 10.18632/oncotarget.21601

[16] Wise DR, Thompson CB. Glutamine addiction: A new therapeutic target in cancer. Trends in Biochemical Sciences. 2010;**35**(8):427-433. DOI: 10.1016/j.tibs.2010.05.003

[17] Eberhardy SR, Farnham PJ. c-Myc mediates activation of the cad promoter via a post-RNA polymerase II recruitment mechanism. The Journal of Biological Chemistry. 2001;**276**(51):48562-48571. DOI: 10.1074/jbc.M109014200

[18] Kim S, Kim DH, Jung WH, Koo JS. Expression of glutamine metabolism-related proteins according to molecular subtype of breast cancer. Endocrine-Related Cancer. 2013;**20**(3):339-348. DOI: 10.1530/ERC-12-0398

[19] Tibbetts AS, Appling DR. Compartmentalization of mammalian folate-mediated one-carbon metabolism. Annual Review of Nutrition. 2010;**30**:57-81. DOI: 10.1146/annurev.nutr.012809.104810

[20] Zeng J, Wang K, Ye F, Lei L, Zhou Y, Chen J, et al. Folate intake and the risk of breast cancer: An up-to-date meta-analysis of prospective studies. European Journal of Clinical Nutrition. 2019;**73**(12):1657-1660. DOI: 10.1038/s41430-019-0394-0

[21] Cavdar Z, Onvural B, Guner G. Arginase in patients with breast cancer. Clinica Chimica Acta; International Journal of Clinical Chemistry. 2003;**338**(1-2):171-172. DOI: 10.1016/j.cccn.2003.09.002

[22] Beloribi-Djefaflia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. Oncogene. 2016;**5**(1):e189. DOI: 10.1038/oncsis.2015.49

[23] Mashima T, Seimiya H, Tsuruo T. De novo fatty-acid synthesis and related pathways as molecular targets for cancer therapy. British Journal of Cancer. 2009;**100**(9):1369-1372. DOI: 10.1038/sj.bjc.6605007

[24] Kuhajda FP. AMP-activated protein kinase and human cancer: Cancer metabolism revisited. International Journal of Obesity. 2008, 2005;**32**(Suppl. 1): S36-S41. DOI: 10.1038/ijo.2008.121

[25] Yan C, Wei H, Minjuan Z, Yan X, Jingyue Y, Wenchao L, et al. The mTOR inhibitor rapamycin synergizes with a fatty acid synthase inhibitor to induce cytotoxicity in ER/HER2-positive breast cancer cells. PLoS One. 2014;**9**(5):e97697. DOI: 10.1371/journal.pone.0097697

[26] Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature. 2012;**486**(7403):400-404. DOI: 10.1038/nature11017

[27] McCarthy AM, Kumar NP, He W, et al. Different associations of tumor PIK3CA mutations and clinical outcomes according to aspirin use among women with metastatic hormone receptor positive breast cancer. BMC Cancer. 2020;**20**:347. DOI: 10.1186/s12885-020-06810-8

[28] Toska E, Osmanbeyoglu HU, Castel P, Chan C, Hendrickson RC, Elkabets M, et al. PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D. Science (New York, N.Y.). 2017;**355**(6331):1324-1330. DOI: 10.1126/science.aah6893

[29] Caino MC, Ghosh JC, Chae YC, Vaira V, Rivadeneira DB, Faversani A, et al. PI3K therapy reprograms mitochondrial trafficking to fuel tumor cell invasion. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**(28):8638-8643. DOI: 10.1073/pnas.1500722112

[30] O'Mahony F, Razandi M, Pedram A, Harvey BJ, Levin ER. Estrogen modulates metabolic pathway adaptation to available glucose in breast cancer cells. Molecular Endocrinology (Baltimore, Md.). 2012;**26**(12):2058-2070. DOI: 10.1210/me.2012-1191

[31] Neeman M, Degani H. Metabolic studies of estrogen- and tamoxifen-treated human breast cancer cells by nuclear magnetic resonance spectroscopy. Cancer Research. 1989;**49**(3):589-594

[32] Ma R, Karthik GM, Lövrot J, Haglund F, Rosin G, Katchy A, et al. Estrogen receptor β as a therapeutic target in breast cancer stem cells. Journal of the National Cancer Institute. 2017;**109**(3):1-14. DOI: 10.1093/jnci/djw236

[33] Maruyama R, Choudhury S, Kowalczyk A, Bessarabova M, Beresford-Smith B, Conway T, et al. Epigenetic regulation of cell type-specific expression patterns in the human mammary epithelium. PLoS Genetics. 2011;**7**(4):e1001369. DOI: 10.1371/journal.pgen.1001369

[34] Girault I, Tozlu S, Lidereau R, Bièche I. Expression analysis of DNA methyltransferases 1, 3A, and 3B in sporadic breast carcinomas. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2003;**9**(12):4415-4422

[35] Huang Y, Nayak S, Jankowitz R, Davidson NE, Oesterreich S. Epigenetics in breast cancer: what's new? Breast Cancer Research: BCR. 2011;**13**(6):225. DOI: 10.1186/bcr2925

[36] Ordway JM, Budiman MA, Korshunova Y, Maloney RK, Bedell JA, Citek RW, et al. Identification of novel high-frequency DNA methylation changes in breast cancer. PLoS One. 2007;**2**(12):e1314. DOI: 10.1371/journal.pone.0001314

[37] Fang F, Turcan S, Rimner A, Kaufman A, Giri D, Morris LG, et al. Breast cancer methylomes establish an epigenomic foundation for metastasis. Science Translational Medicine. 2011;**3**(75):75ra25. DOI: 10.1126/scitranslmed.3001875

[38] Abdel-Hafiz HA, Horwitz KB. Role of epigenetic modifications in luminal breast cancer. Epigenomics. 2015;**7**(5):847-862. DOI: 10.2217/epi.15.10

[39] Holm K, Hegardt C, Staaf J, Vallon-Christersson J, Jönsson G, Olsson H, et al. Molecular subtypes of breast cancer are associated with characteristic DNA methylation patterns. Breast Cancer Research: BCR. 2010;**12**(3):R36. DOI: 10.1186/bcr2590

[40] Sawan C, Herceg Z. Histone modifications and cancer. Advances in Genetics. 2010;**70**:57-85. DOI: 10.1016/B978-0-12-380866-0.60003-4

[41] Marson CM. Histone deacetylase inhibitors: Design, structure-activity

relationships and therapeutic implications for cancer. Anti-Cancer Agents in Medicinal Chemistry. 2009;**9**(6):661-692. DOI: 10.2174/187152009788679976

[42] Jenuwein T, Allis CD. Translating the histone code. Science (New York, N.Y.). 2001;**293**(5532):1074-1080. DOI: 10.1126/science.1063127

[43] Woo J, Kim HY, Byun BJ, Chae CH, Lee JY, Ryu SY, et al. Biological evaluation of tanshindiols as EZH2 histone methyltransferase inhibitors. Bioorganic & Medicinal Chemistry Letters. 2014;**24**(11):2486-2492. DOI: 10.1016/j.bmcl.2014.04.010

[44] Luo XG, Zou JN, Wang SZ, Zhang TC, Xi T. Novobiocin decreases SMYD3 expression and inhibits the migration of MDA-MB-231 human breast cancer cells. IUBMB Life. 2010;**62**(3):194-199. DOI: 10.1002/iub.288

[45] Lim S, Janzer A, Becker A, Zimmer A, Schüle R, Buettner R, et al. Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology. Carcinogenesis. 2010;**31**(3):512-520. DOI: 10.1093/carcin/bgp324

[46] Hurst DR, Edmonds MD, Scott GK, Benz CC, Vaidya KS, Welch DR. Breast cancer metastasis suppressor 1 up-regulates miR-146, which suppresses breast cancer metastasis. Cancer Research. 2009;**69**(4):1279-1283. DOI: 10.1158/0008-5472.CAN-08-3559

[47] Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD, et al. Endogenous human microRNAs that suppress breast cancer metastasis. Nature. 2008;**451**(7175):147-152. DOI: 10.1038/nature06487

[48] Mansoori B, Mohammadi A, Ghasabi M, Shirjang S, Dehghan R, Montazeri V, et al. miR-142-3p as tumor suppressor miRNA in the regulation of tumorigenicity, invasion and migration of human breast cancer by targeting Bach-1 expression. Journal of Cellular Physiology. 2019;**234**(6):9816-9825. DOI: 10.1002/jcp.27670

[49] Fan M, Sethuraman A, Brown M, Sun W, Pfeffer LM. Systematic analysis of metastasis-associated genes identifies miR-17-5p as a metastatic suppressor of basal-like breast cancer. Breast Cancer Research and Treatment. 2014;**146**(3):487-502. DOI: 10.1007/s10549-014-3040-5

[50] Li WJ, Xie XX, Bai J, Wang C, Zhao L, Jiang DQ. Increased expression of miR-1179 inhibits breast cancer cell metastasis by modulating notch signaling pathway and correlates with favorable prognosis. European Review for Medical and Pharmacological Sciences. 2018;**22**(23):8374-8382. DOI: 10.26355/eurrev_201812_16535

[51] Connolly EC, Van Doorslaer K, Rogler LE, Rogler CE. Overexpression of miR-21 promotes an in vitro metastatic phenotype by targeting the tumor suppressor RHOB. Molecular Cancer Research: MCR. 2010;**8**(5):691-700. DOI: 10.1158/1541-7786.MCR-09-0465

[52] Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science. 1989;**244**:707-712

[53] Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nature Reviews. Molecular Cell Biology. 2001;**2**:127-137

[54] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL.

Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;**235**(4785):177-182

[55] Redmond AM, Omarjee S, Chernukhin I, Le Romancer M, Carroll JS. Analysis of HER2 genomic binding in breast cancer cells identifies a global role in direct gene regulation. PLoS One. 2019;**14**(11):e0225180

[56] Giri DK, Ali-Seyed M, Li LY, Lee DF, Ling P, Bartholomeusz G, et al. Endosomal transport of ErbB-2: Mechanism for nuclear entry of the cell surface receptor. Molecular and Cellular Biology. 2005;**25**(24):11005-11018

[57] Beguelin W, Diaz Flaque MC, Proietti CJ, Cayrol F, Rivas MA, Tkach M, et al. Progesterone receptor induces ErbB-2 nuclear translocation to promote breast cancer growth via a novel transcriptional effect: ErbB-2 function as a coactivator of Stat3. Molecular and Cellular Biology. 2010;**30**(23):5456-5472

[58] Diaz Flaque MC, Vicario R, Proietti CJ, Izzo F, Schillaci R, Elizalde PV. Progestin drives breast cancer growth by inducing p21(CIP1) expression through the assembly of a transcriptional complex among Stat3, progesterone receptor and ErbB-2. Steroids. 2013;**78**(6):559-567

[59] Li LY, Chen H, Hsieh YH, Wang YN, Chu HJ, Chen YH, et al. Nuclear ErbB2 enhances translation and cell growth by activating transcription of ribosomal RNA genes. Cancer Research. 2011;**71**(12):4269-4279

[60] Wang SC, Lien HC, Xia W, Chen IF, Lo HW, Wang Z, et al. Binding at and transactivation of the COX-2 promoter by nuclear tyrosine kinase receptor ErbB-2. Cancer Cell. 2004;**6**(3):251-261

[61] Dillon MF, Stafford AT, Kelly G, Redmond AM, McIlroy M, Crotty TB, et al. Cyclooxygenase-2 predicts adverse effects of tamoxifen: A possible mechanism of role for nuclear HER2 in breast cancer patients. Endocrine-related Cancer. 2008;**15**(3):745-753

[62] Isola J, Chu L, DeVries S, Matsumura K, Chew K, Ljung BM, et al. Genetic alterations in ERBB2-amplified breast carcinomas. Clinical Cancer Research. 1999;**5**:4140-4145

[63] Ellsworth RE, Ellsworth DL, Patney HL, et al. Amplification of HER2 is a marker for global genomic instability. BMC Cancer. 2008;**8**:297

[64] LeBleu VS, O'Connell JT, Gonzalez Herrera KN, Wikman H, Pantel K, Haigis MC, et al. PGC-1α mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. Nature Cell Biology. 2014;**16**:992-1003

[65] Dupuy F, Bastien TS, Andrzejewski S, Pierre JS, Jones RG, Siegel PM, et al. PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. Cell Metabolism. 2015;**22**:577-589

[66] Gandhi N, Das GM. Metabolic reprogramming in breast cancer and its therapeutic implications. Cell. 2019;**8**(2):89

[67] Zhang D, Tai LK, Wong LL, Chiu LL, Sethi SK, Koay ES. Proteomic study reveals that proteins involved in metabolic and detoxification pathways are highly expressed in HER-2/neu-positive breast cancer. Molecular & Cellular Proteomics. 2005;**4**:1686-1696

[68] Zhao YH, Zhou M, Liu H, Ding Y, Khong HT, Yu D, et al. Upregulation of lactate dehydrogenase a by ErbB2

through heat shock factor 1 promotes breast cancer cell glycolysis and growth. Oncogene. 2009;**28**:3689-3701

[69] Walsh AJ, Cook RS, Manning HC, Hicks DJ, Lafontant A, Arteaga CL, et al. Optical metabolic imaging identifies glycolytic levels, subtypes, and early-treatment response in breast cancer. Cancer Research. 2013;**73**:6164-6174

[70] O'Neal J, Clem A, Reynolds L, DoughertyS,Imbert-FernandezY,TelangS, et al. Inhibition of 6-phosphofructo-2-kinase (PFKFB3) suppresses glucose metabolism and the growth of HER2+ breast cancer. Breast Cancer Research and Treatment. 2016;**160**:29-40

[71] Ding Y, Liu Z, Desai S, Zhao Y, Liu H, Pannell LK, et al. Receptor tyrosine kinase ErbB2 translocates into mitochondria and regulates cellular metabolism. Nature Communications. 2012;**3**:1271

[72] Cao MD, Lamichhane S, Lundgren S, Bofin A, Fjosne H, Giskeodegard GF, et al. Metabolic characterization of triple negative breast cancer. BMC Cancer. 2014;**14**:941

[73] Holloway RW, Marignani PA. Targeting mTOR and glycolysis in HER2-positive breast cancer. Cancers (Basel). 2021;**13**(12):2922

[74] Nami B, Ghanaeian A, Black C, Wang Z. Epigenetic silencing of HER2 expression during epithelial-mesenchymal transition leads to trastuzumab resistance in breast cancer. Life (Basel). 2021;**11**(9):868

[75] Karsli-Ceppioglu S, Dagdemir A, Judes G, et al. The epigenetic landscape of promoter genome-wide analysis in breast cancer. Scientific Reports. 2017;**7**:6597

[76] Fuino L, Bali P, Wittmann S, Donapaty S, Guo F, Yamaguchi H,

et al. Histone deacetylase inhibitor LAQ824 downregulates her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. Molecular Cancer Therapeutics. 2003;**2**:971-984

[77] Angelousi A, Alexandraki KI, Kyriakopoulos G, Tsoli M, Thomas D, Kaltsas G, et al. Neoplastic metastases to the endocrine glands. Endocrine-Related Cancer. 2020;**27**(1):R1-R20

[78] Ballinger TJ, Meier JB, Jansen VM. Current landscape of targeted therapies for hormone-receptor positive, HER2 negative metastatic breast cancer. Frontiers in Oncology. 2018;**8**:308

[79] Watson SS, Dane M, Chin K, Tatarova Z, Liu M, Liby T, et al. Microenvironment-mediated mechanisms of resistance to HER2 inhibitors differ between HER2+ breast cancer subtypes. Cell Systems. 2018;**6**(3):329-342.e6

[80] American Cancer Society. Triple-negative breast cancer: Details, diagnosis, and signs. In: Triple-Negative Breast Cancer. 2022. Available from: https://www.cancer.org/cancer/breast-cancer/about/types-ofbreast-cancer/triplenegative

[81] Centers for Disease Control and Prevention. Triple-negative breast cancer. In: Triple-Negative Breast Cancer. 2021. Available from: https://www.cdc.gov/cancer/breast/triple-negative.htm

[82] Neophytou C, Boutsikos P, Papageorgis P. Molecular mechanisms and emerging therapeutic targets of triple-negative breast cancer metastasis. Frontiers in Oncology. 2018;**8**. DOI: 10.3389/fonc.2018.00031

[83] van Londen G, Beckjord E, Dew M, Cuijpers P, Tadic S, Brufsky A.

Breast cancer survivorship symptom management: Current perspective and future development. Breast Cancer Management. Jan 2013;**2**(1):71-81. DOI: 10.2217/bmt.12.63. PMID: 23814614; PMCID: PMC3693468

[84] Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, et al. Refinement of triple-negative breast cancer molecular subtypes: Implications for neoadjuvant chemotherapy selection. PLoS One. 16 Jun 2016;**11**(6):e0157368. DOI: 10.1371/journal.pone.0157368. PMID: 27310713; PMCID: PMC4911051

[85] Fultang N, Chakraborty M, Peethambaran B. Regulation of cancer stem cells in triple negative breast cancer. Cancer Drug Resistance. 2021. DOI: 10.20517/cdr.2020.106

[86] Steeg P. Targeting metastasis. Nature Reviews Cancer. 2016;**16**:201-218. DOI: 10.1038/nrc.2016.25

[87] Petri BJ, Klinge CM. Regulation of breast cancer metastasis signaling by miRNAs. Cancer and Metastasis Reviews. 2020;**39**(3):837-886. DOI: 10.1007/s10555-020-09905-7

[88] Kim J, Yao F, Xiao Z, Sun Y, Ma L. MicroRNAs and metastasis: Small RNAS play big roles. Cancer and Metastasis Reviews. 2017;**37**(1). DOI: 10.1007/s10555-017-9712-y

[89] Keklikoglou I, Koerner C, Schmidt C, Zhang JD, Heckmann D, Shavinskaya A, et al. MicroRNA-520/373 family functions as a tumor suppressor in estrogen receptor negative breast cancer by targeting NF-κB and TGF-β signaling pathways. Oncogene. 2012;**31**:4150-4163. DOI: 10.1038/onc.2011.571

[90] Xie W, Du Z, Chen Y, Liu N, Zhong Z, Shen Y, et al. Identification of metastasis-associated genes in triple-negative breast cancer using weighted gene co-expression network analysis. Evolutionary Bioinformatics. 2020;**16**:117693432095486. DOI: 10.1177/1176934320954868

[91] Howard SR. The genetic basis of delayed puberty. Frontiers in Endocrinology. 2019;**10**. DOI: 10.3389/fendo.2019.00423