Chapter

Molecular Genetics of Metastatic Breast Cancer

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Abstract

Breast cancer is the most common form of cancer in women. Breast cancer has a heterogeneous etiology. Genetic and environmental factors contribute to the pathogenesis and progression of breast cancer. Various genes as proliferation and nuclear factors have been identified in breast cancer. Therefore, the genetic component of patients is important in determining disease behavior, response to anticancer therapeutics, and patient survival. Prognosis of breast cancer is associated with potential metastatic properties of primary breast tumors. Metastasis is the leading cause of death in patients with breast cancer. Therefore, it is important to understand the mechanisms underlying the development of distant metastases to specific regions and has clinical value. Metastasis shows an organ-specific spread pattern and occurs with a series of complex and multistep events associated with each other, such as angiogenesis, invasion, migration-motility, extravasation, and proliferation. Breast cancer often metastasizes to the bone, liver, brain, and lungs. Metastasis may develop years after successful primary treatment. The metastatic process will become clear, as information about molecules and genes associated with metastases increases, and this is extremely important for cancer treatment.

Keywords: breast cancer, metastasis, genes, pathways, organs

1. Introduction

Breast cancer, which is one of the most common malignant diseases of women worldwide, is a heterogeneous disease with unknown pathogenesis. Genetic and environmental factors contribute to the pathogenesis and progression of breast cancer. Although an improvement has recently been detected in the diagnosis and treatment of breast cancer compared with other cancers, its contribution to survival was inadequate.

Breast cancer-associated death or survival is associated with the potential metastatic features of the primary breast tumors. Metastatic disease is the leading cause of death in breast cancer patients. Distant metastasis develops in ~20–30% of the early-stage breast cancer patients. Approximately 90% of deaths result from the complications due to recurrent or metastatic diseases. Therefore, it is very important to understand the underlying mechanisms in the development of distant metastases to specific regions. Metastases may show an organ-specific dissemination pattern. Metastasis may develop years after successful primary treatment. Metastasis frequently develops in the bone, liver, brain, and lungs in breast cancer.

Identification of the molecules, and genes associated with metastasis, and clarification of the contribution of these molecules to metastatic process are important

for the treatment of cancer. Metastasis is the dissemination of the cancer cells from their primary region to different tissues and organs in the body. Metastasis develops with a series of complex and multistep chains of events such as angiogenesis, invasion, migration-motility, extravasation, and proliferation.

The anomalies of different genes as *BRCA1*, *BRCA2*, *MYC*, *TP53*, *RB1*, *JUN*, and *CDK2A* which have roles in cell proliferation are detected in breast cancer [1]. Therefore, performing the genetic and molecular screenings of patients is important for the identification of the behavior of disease, the anticancer therapeutic response, and the survival.

Different breast cancer cellular subtypes in primary breast cancer tissue metastasize in relation to their target organ. The route of metastasis is generated with the interaction of this different subtype cells and microenvironment of the tumor and with the organ they will locate, and this is named as "organotrophic metastasis."

Understanding the molecular mechanism of organotrophic metastasis is very important for biological indicator prediction, developing the innovative therapeutic strategies, and for improving the survival. Development of metastasis in distant regions is associated with the interaction between the tumor cells and host microenvironment. Before the initiation of tumor dissemination, the host microenvironment is modified to support the tumoral growth, in other words to create a pre-metastatic niche (PMN). PMN is organized with the factors secreted from tumor cell with the changes in the host cell metabolism and microenvironment. In addition, tumor cells also interact with the extracellular matrix (ECM) of the host tissue to facilitate metastasis.

Generally, breast cancer is classified as in situ carcinoma and invasive carcinoma in a simple way, and most breast cancers are invasive. More than 80% of invasive breast cancers may be investigated in two different subgroups as invasive ductal carcinoma (IDC), and some breast cancers may be investigated as invasive lobular carcinoma (ILC). Organ preference of metastasis in ILC and IDC is significantly distinct. Invasive ductal carcinomas do metastasis to the lungs, distant lymphatic glands, and central nervous system (CNS); however, ILC is known to do threefold higher metastasis to the peritone, gastrointestinal system, and ovaries [2].

Breast cancer has a tendency to do metastasis on the bone, liver, lung, and distant lymphatic glands. The most common metastasis type is the bone metastases detected in 70% of metastatic breast cancer patients [1]. The second most common metastasis region was the liver with ~30%, and the brain was reported as the third most common metastasis region with a rate of 10–30% [1].

The most common metastatic region in all subtypes except basal-like tumors is the bone. Luminal B, HER2+/ER/PR+ and HER2+/ER/PR, tumors do more metastasis to the brain, liver, lungs, and bone than the luminal A tumors. Basal-like tumors do higher rates of the brain, lungs, and distant lymphatic node metastasis; however, the liver and bone metastases are less frequently detected in basal-like tumors [3]. Although triple negative breast cancer (TNBC) tumors show a metastatic ratio similar to non-basal tumors, TNBC tumors have less liver metastasis than the non-basal tumors [1].

Some molecules may have different roles in different metastasis regions in accordance with their content. Although transforming growth factor beta (*TGF-β*) promotes the lung metastasis of breast cancer, its interaction with Src signaling pathway may cause bone metastasis [4]. In cancer cells with insulin-like growth factor (*IGF1*) and *IGF1* receptor (*IGF1R*), bone metastasis shows higher expression than the cancer cells with brain metastasis [5]. EGFR ligands and cyclooxygenase 2 (*COX2*) were reported to be associated with lung metastasis and, however, were reported to be not associated with bone or liver metastasis [6].

Wnt-1-inducible-signaling pathway protein1 (*WISP-1*) and *CCN4* are heparin-binding glycoproteins of the CCN protein family that are rich in cysteine. These

proteins are expressed in various inner organs such as the lung, kidney, and spleen. *WISP-1* binds to *BMP-2* and increases the mesenchymal cellular proliferation and osteoblastic differentiation. *WISP-1* was reported to be associated with the increased metastasis risk among early-stage ER-positive lymphatic node-negative breast cancer patients [7]. Therefore, future studies will demonstrate whether genetic factors associated with WISP-1 and EXT1 genes may show metastasis risk or may be used in identification of metastasis. In addition, the increase of WISP-1 expression was proven to be associated with the pathogenesis of the primary lung cancers. Although the possibility of *WISP-1* to be used as a prognostic indicator for lung metastasis of breast cancer was suggested, it was not clarified yet whether *WISP-1* was a tumor stimulant or a tumor suppressor.

Breast cancer cells are detected to highly express the chemokine receptors *CXCR4* and *CCR7* genes in the studies investigating the contribution of chemokine receptors to organ-specific metastases. Chemokine receptor-specific ligands *CXCL12* and *CCL21* were demonstrated to be highly expressed in the organs to which breast tumors do metastasis such as the lymph nodes, lungs, liver, and bone marrow [8]. In addition, the blockade of *CXCR4* gene in experimental animal models was demonstrated to inhibit the metastasis of breast cancer cells. The activation of the RAS/mitogen-activated protein kinase (MAPK) with chemokine signaling pathway causes changes in primary cancer cells such as changes in the intracellular actin molecule polymerization, development of pseudopodia, and increased cellular motility, cellular migration, and tissue invasion. Any of these changes contribute to the development of organ-specific metastasis by contributing to the survival, metastasis, and vitality ability of cancer cells.

2. Metastasis-associated signal transduction pathways and genes

2.1 p38/MAPK pathway

p38/MAPK signal transduction pathway increases the breast carcinoma vascularization and growth by promoting the expression and accumulation of protumorigenic factors.

The inactivation of the p38/MAPK signaling pathway was provided by the expression of the kinase-inactive mutant (dn-p38) of p38/MAPK14 in metastatic breast cancer cells in the studies, and with the deterioration of the tumor p38/MAPK signal, the development of breast cancer and metastasis ability was shown to decrease in breast carcinoma xenografts [9]. The conducted kinase-inactive mutant significantly decreased the dn-p38, tumor blood vessel density, and lumen dimensions. p38 controls the expression of the pro-angiogenic extracellular factors such as matrix protein fibronectin, cytokine, vascular endothelial growth factor A (*VEGFA*), and *IL8*. p38/MAPK signal transduction was demonstrated to increase the tumoral growth, and vascularization in addition to increasing the expressions of tumor-associated fibroblasts, and pro-angiogenic factors. All these effects were suppressed by the dn-p38 kinase-inactive mutant. The data analyses showed that p38 had higher expression in breast cancers which was an indicator of recurrence and poor prognosis. The activation of the p38/MAPK signaling pathway in the tumor increased the development of breast cancer and metastasis. *p38* contributes to the vascularization of carcinoma by facilitating the expression and accumulation of the pro-angiogenic factors. In conclusion, all these results suggested that all the genes which have a role in p38/MAPK pathway might be a therapeutic target against tumor vascularization and metastasis.

Tumor microenvironment (TME) is an important factor in cancer progression, recurrence, and response to treatment. TME blood vessels consist of stromal

cells (fibroblasts, adipocytes) and infiltrating immune cells. Myeloid cells stimulate the tumor vascularization and metastasis by secreting metalloproteinase *MMP9/gelatinase-B* cells which increase the gathering of endothelial cells and pericytes. In addition to myeloid cells, *MMP9* is also produced by the breast carcinoma cells, and the *MMP9* destruction in carcinoma cells significantly decreases tumor vascularization [10]. Therefore, all three cellular components of the TME of breast contribute to the tumor vascularization by interacting with *MMP9*. p38/MAPK signal contributes to the development of breast cancer and metastasis by increasing the tumor cell invasiveness and tumor vascularization.

MMP9 that has a role in tumor angiogenesis and intratumoral vascularization-associated *ICAM1* works correlated with p38/MAPK signal. *ICAM1* is also suggested as a target in triple negative breast cancer (TNBC) [11]. The inhibition of p38/MAPK signal affects the TNF-induced *ICAM1* expression or the induction of *MMP9* by the cytokines *TGF-β* and TNF.

The deterioration of p38/MAPK signal causes no decrease in the expression of *MMP9* and *ICAM1* that are secreted by tumor cells. p38/MAPK signal contributes to fibronectin expression by responding to cytokines and tumor-fibroblast interactions [9].

p38/MAPK induces the expression of pro-angiogenic cytokines that include *VEGFA*, *IL8*, and *HBEGF* in addition to inducing an extracellular matrix protein fibronectin. *TAK1* controls the expression of MMP9 which releases *VEGF* and activates the *IL8* (**Figure 1**). Pro-angiogenic cytokines increase the tumoral growth by stimulating the tumoral vascularization.

p38/MAPK affects the development and metastasis of breast cancer by changing the tumor microenvironment of p38/MAPK signal. The inactivation of p38/MAPK signal in breast cancer cells decreases the growth of tumor xenografts and metastasis. Tumoral and stromal cells in breast TME stimulate the cytokine-mediated p38/MAPK signal which increases the expression of the pro-angiogenic and pro-invasive factors such as *VEGFA*, *IL8*, *IL6*, *HBEGF*, and fibronectin. p38/MAPK which affects

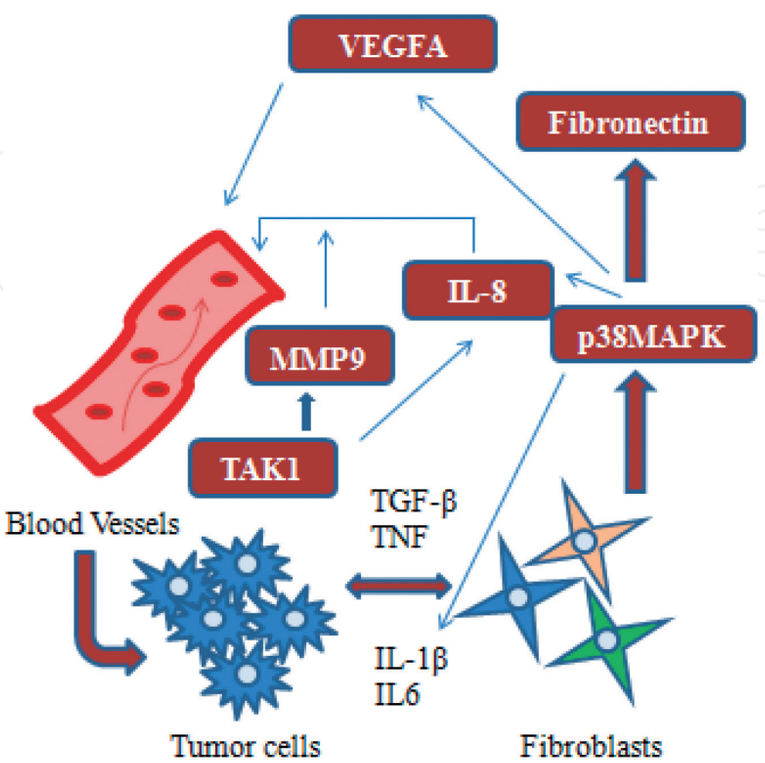

Figure 1.
The role of p38/MAPK in the regulation of tumor angiogenesis in breast cancer.

the vascular structure and stroma of tumor is detected to be definitely a potential target for anticancer treatment. Researchers suggested that anti-p38 drugs were a new therapeutic option in the treatment of breast cancer including metastatic disease [9].

2.2 Tumor endothelial marker 8 (TEM8)

Tumor endothelial marker (*TEM8*) was first discovered in the human tumor endothelial and was associated with tumor angiogenesis. TEM8 also known as Anthrax toxin receptor 1 (*ANTXR1*) is highly regulated in tumor endothelial and is expressed in breast cancer. *TEM8* was demonstrated to be required for tumoral growth and angiogenesis [12]. The role of *TEM8* in angiogenesis is organized with the regulation of downstream VEGF signal with its interaction with vascular endothelial growth factor receptor 2 (*VEGFR2*). Primary tumor development and metastasis are highly dependent on angiogenesis. Because the tumor cannot grow more than a few millimeters unless new blood vessels that will provide the oxygen and nutrients to tumor tissue are generated. The extravasation and dissemination of metastatic cells out of the vessel are facilitated and accelerated due to the leaky structure of the rapid developing tumor vessel network during tumor angiogenesis. Therefore, treatments targeting *TEM8* can differentiate the physiologic and pathological angiogenesis and can prevent the cancer progression without causing serious adverse effects. Due to this feature, *TEM8* is suggested to be a new possible therapeutic target in inhibiting the metastasis.

The destruction of *TEM8* in osteosarcoma cells causes the decrease of the cell proliferation [13]. *TEM8* interacts with the lipoprotein receptor associated protein 6 (*LRP6*) and regulates the downstream signaling of Wnt which is a protein that induces both the cellular proliferation and migration. *TEM8* was reported to regulate metastasis and a new molecule specific for metastasis by contributing the breast cancer stem cells (BCSC) and tumor growth with activating the Wnt signal with collagen VI [14]. *TEM8* is associated with invasive and aggressive phenotype in breast cancer. In addition, *TEM8* expression was demonstrated to be highly expressed in the tumor tissues of breast cancer patients compared to the normal tissues [14].

TEM8 expressed by cancer cells causes the development of angiogenesis by affecting the cancer cell proliferation and endothelial cell migration. *TEM8* knockout (KO) cells were generated using CRISPR/Cas9, *TEM8* expression was demonstrated to significantly disappear, and *TEM8* was inhibited in the studies investigating the association of *TEM8* with metastasis (**Figure 2**). Thus, angiogenesis decreased in tumor cells, and metastasis ability of *TEM8* significantly

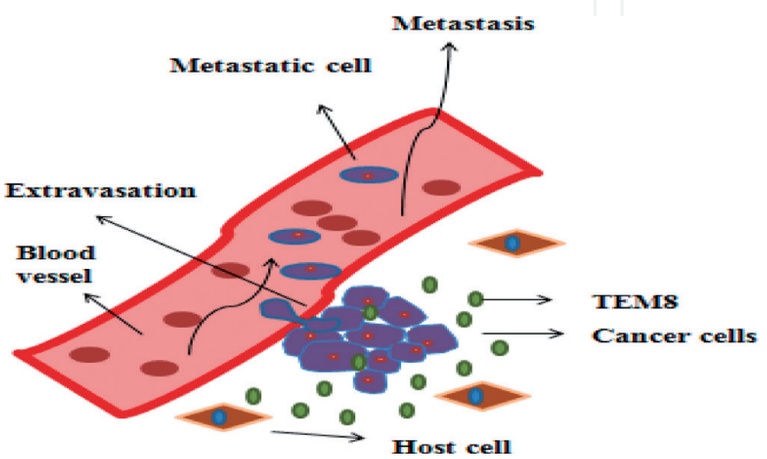

Figure 2.
The effect of TEM8 in breast cancer metastasis.

degraded with the deletion in cancer cells. Cancer cell proliferation, angiogenesis, and metastases are blocked with the prevention of cell cycle and the expression of the kinetochore-associated genes with the inhibition of *TEM* [15]. Cancer cells are known to secrete the pro-angiogenic signals such as *VEGFA* and open the angiogenic lock by affecting the tumor microenvironment. *TEM8* is known to work in cooperation with other factors such as *VEGF* for promoting endothelial cell migration and angiogenesis. In conclusion, *TEM8* expression is higher in tumor cells than in normal cells. Studies conducted using *TEM8* knockout metastatic breast cancer cell lines designed with CRISPR/Cas9 emphasize the role of *TEM8* in cancer development, tumor angiogenesis, and local metastasis. All these studies reveal the potential of *TEM8* as a therapeutic target for combating the disease; however, more clinical studies are required for developing the *TEM8*-targeted therapies [15].

2.3 APOBEC3B gene

Another important molecule in the development of metastatic potential of breast cancer is *APOBEC3B*. High level of *APOBEC3B* mRNA expression was demonstrated to be a significant prognostic biological indicator demonstrating the poor prognosis of breast cancer in ER-positive primary breast cancer cases. In addition, this molecule in distant metastasis regions was demonstrated to be highly expressed than the levels in regional lymph node metastases. This showed that *APOBEC3B* not only in the primary tumor stage has a role in the development of different metastatic stages of breast cancer. In conclusion, *APOBEC3B* causes the progression of metastatic breast cancer [16]. Therefore, the identification of different expression levels of *APOBEC3B* suggests that it carries a biological marker feature that may show a different metastatic stage and may be used in the identification of the metastasis stages in future.

3. Metastasis of breast cancer to different organs

3.1 Lymph node metastasis

Lymph node metastasis shows that distant metastasis risk is higher. The absence of lymph node metastases is associated with lower metastasis risk; however, the presence of more than four lymph node metastases is the precursor that distant metastasis risk is significantly higher. Distant tumor metastasis develops through axillary lymphoid nodes (ALD) and blood circulation. Therefore, lymph nodes are used as an indicator of the metastasis ability of tumor cells. There is an association between the tumor size and the rate of lymph node metastasis.

CCN proteins which have oncogenic functions in breast cancer mainly consist of *CCN1* and *CCN2*. *CCN1* protein is expressed in ~30% of breast cancers particularly in estrogen receptor (ER)-positive HER-2-negative tumors compared with the normal breast tissues. Higher *CCN1* expression is associated with lymph node metastasis and poor prognosis in breast cancer patients. *CCN1* increases the breast tumor vascularization and causes metastasis with Hg signaling [17]. In addition, *CCN1* has a regulatory role in fibroblast production by affecting MMP-1 for increasing the breast cancer cell migration and invasion. CCN4 expression is associated with lymph node metastasis and poor prognosis.

3.2 Bone metastasis

The common cause of morbidity and mortality in most advanced stage breast cancer patients is the development of osteolytic bone metastasis. The most

frequently detected area of metastasis in metastatic breast cancer is the bone and constitutes 70% of the metastases. Most bone metastases detected in breast cancer are associated with osteolytic-type metastatic lesions owing to the osteoclast-mediated bone resorption. Although all subtypes of breast cancer have a tendency of bone metastases, luminal subtype tumors develop higher bone metastases (80.5%) than the basal-like (41.7%) and HER2+ tumors (55.6%) [18].

Tumor cells demonstrate different reactions in accordance with the environment in the new organ such as gene expression, growth ability, and response to treatment. Therefore, any of the breast cancer cell reaching to the bone may promote the excessive growth in molecular interaction with osteoblasts and osteoclasts. The molecules produced by cancer cells or with the parathyroid hormone-associated protein in the bone microenvironment and converting growth factor β (*TGF-β*) mediate this growth. The elimination of the tumor suppressor feature of *TGF-β* is suggested to stimulate the tumor invasion and metastasis [19]. Cytokines, chemokines, and other growth factors support the development of bone metastasis. Prometastatic cytokine *TGF-β*, osteolytic angiogenic factors interleukin-11 (*IL11*), and CTGF expression are accepted as the molecules that increase the osteolytic metastatic activity. Although *SMAD4* is a tumor suppressor which inhibits the tumor cell proliferation, it is an osteolytic metastasis promoter which binds the *TGF-β* signal to the following *IL11* induction [20]. *SMAD4* activates *VEGF* and *CXC* chemokine receptor 4 (*CXCR4*) to induce the bone metastasis in breast cancer through *HIF-lα* and *TGF-β* signal.

Some cancer cells in the primary tumor accumulate additional genetic changes which lead to bone metastasis. This causes invasion and colonization of tumor cells to the bone matrix. The destruction of the bone matrix with tumor cells facilitates the metastasis by the TGF and metastasis genes responding to TGF causing the increase of *CTGF* and *IL11* expression. *IL11*, *CTGF*, *CXCR4*, and *MMP-1* are the four most effective genes that are overexpressed in bone metastasis. Another effective gene is the protein osteopontin (OPN) which has various functions including the stimulating ability of the bone matrix to attach to the osteoclast. This protein is continuously overexpressed in metastatic cells. The genes effective in bone metastasis affect the tumor microenvironment toward metastasis. The overexpression of these genes develops the osteolytic bone metastasis. *IL11* is a strong osteoclast inducer which is synthesized by the progenitor cells in the bone marrow [17]. The in vivo testing of *IL11*-transfected MDA-MB-231 for metastatic activity of metastatic breast cancer cell line showed that the single expression of *IL11* did not significantly increase the metastasis. Therefore the presence of other genes in cooperation with IL11 in bone metastasis and their investigation were suggested [17]. *IL11* and *OPN* significantly increased the osteolytic bone metastasis by increasing the osteoclast function. *MMP-1* alone or in combination with *IL11* and *OPN* is another important molecule in the development of bone metastasis.

Because *TGF-β* is abundantly stored in the bone matrix, *TGF-β* that is secreted during osteolysis stimulates the metastatic breast cancer. *TGF-β* increases the *IL11* and *CTGF* expressions which are already higher in metastasis. The significantly overexpressed genes in bone metastasis encode the cell surface and secreting proteins which have functions that could possibly change the host tissue environment, each promoting the formation of osteolytic bone lesions.

Figure 3 demonstrates the functioning between the *CXCR4* gene responsible in bone marrow extravasation, *MMP-1* and *ADAMTS1* genes having roles of proteolysis and also *FGF5* and *CTGF* genes that are known to be expressed in angiogenesis, and IL11 genes which have a role in osteoclastogenesis.

Primary breast tumor develops with the accumulation of oncogenic mutations from normal breast epithelium. The increased expression of gene classes that

facilitate metastasis to different organs among tumor cells enables the invasion of the bone matrix, colonization of metastatic tumor cell, and destruction of the bone matrix [21].

CCN protein family consists of six members as *CCN1* (*Cyr61*), *CCN2* (*CTGF*), *CCN3* (*Nov*), *CCN4* (*WISP-1*), *CCN5* (*WISP2*), and *CCN6* (*WISP3*) which have central roles in development, inflammation, and tissue repair [22]. In addition, CCN proteins have roles in various pathological cases by organizing the extracellular signals in the cellular environment. In MDA-MB-231 metastatic breast cancer cell line, *CCN3* reorganizes the actin cytoskeleton and increases the cell trafficking by activating the GTPase *Rac1* [23]. *CCN3* was demonstrated to increase the bone metastasis in the studies conducted in metastatic breast cancer cell line [23]. This significant effect of *CCN3* in metastasis was reported to deteriorate the osteoblast differentiation and provided a favorable environment for osteolytic breast cancer bone metastasis owing to supporting the osteoclastogenesis [23].

One of the overexpressed genes in bone-specific metastasis is the *NAT1* (N-acetyltransferase-1) and is a potential biological indicator for breast cancer.

3.3 Liver metastasis

The liver is the most common metastatic region for cancers and represents the second organ where breast cancer metastasis occurs. The development of liver metastasis in breast cancer patients is associated with Wnt signal and Ki67 signal independent of beta-catenin and an indicator of poor prognosis.

CXCR4 is the most common chemokine receptor that mediates the initiation of liver metastases. In addition, the dysregulation of cell adhesion molecules *N-cadherin* and *E-cadherin* was demonstrated to contribute to liver metastases in breast cancer (**Figure 4**). Breast cancer cells with higher *N-cadherin* level develop liver metastasis. *E-cadherin* which inhibits the metastasis was found lower in breast cancer cells with liver metastasis [24].

Although *N-cadherin* increases the liver metastasis, in normal conditions *E-cadherin* suppresses the development of liver metastasis. In addition, *IL-6*

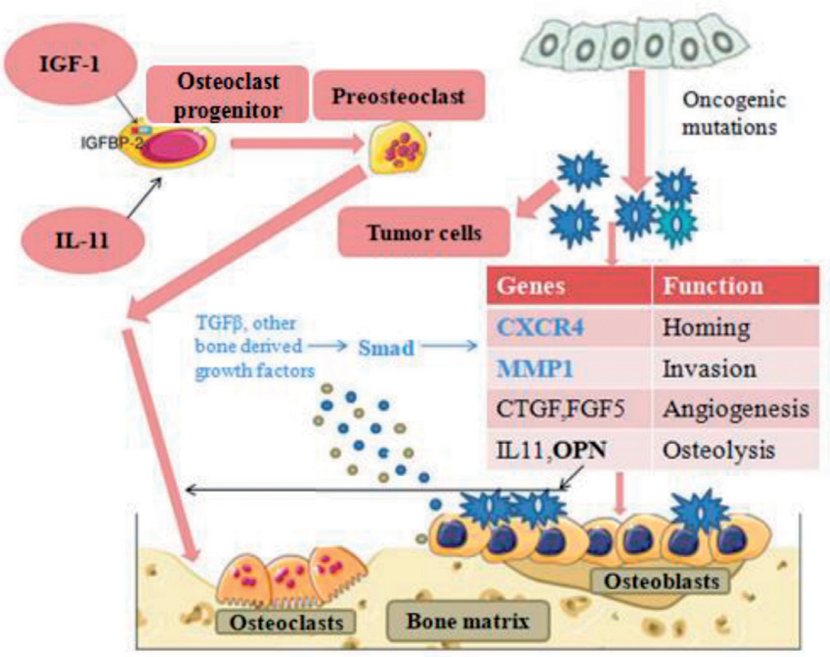

Figure 3.
The molecular mechanisms that are mediated by the genes effective in breast cancer-associated bone metastasis.

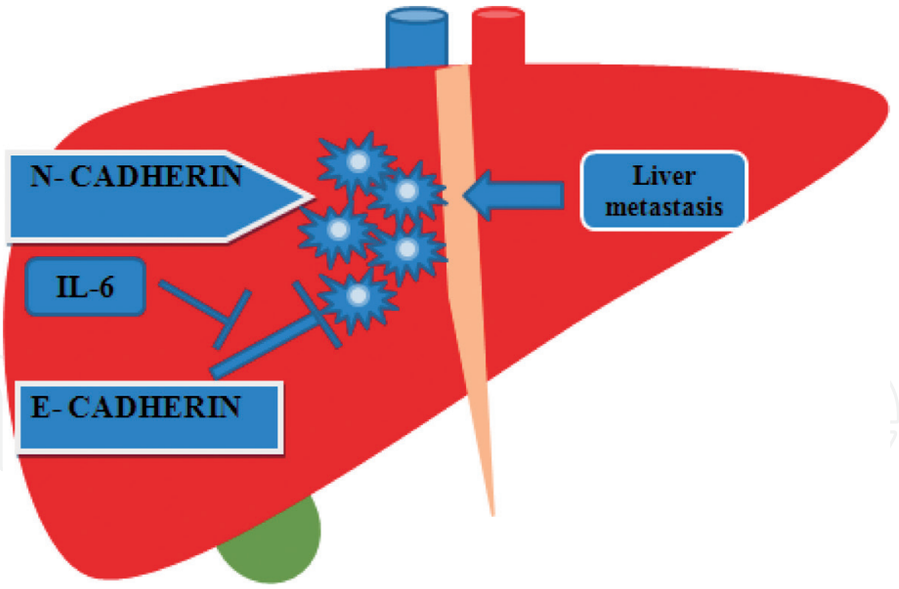

Figure 4.
Regulation of cell adhesion molecules in liver metastasis with N-cadherin and E-cadherin.

expression in liver metastasis of breast cancer facilitates the development of liver metastasis by inhibiting the *E-cadherin* expression [24].

Metastasis is a multistep procedure which is responsible for most cancer-associated deaths and is affected by both cell-cell or cell-matrix interactions and tumor microenvironment (vascularization, etc.).

Clinically, low oxygen level (hypoxia) is known to be associated with metastasis [17]. Lysyl oxidase (*LOX*) expression is both associated with tumor suppression and tumor progression, and its role in tumorigenesis changes in accordance with the cellular location, cell type, and transformation. *LOX* expression is regulated by the hypoxia-inducible factor (*HIF*). Mostly distant metastasis is detected, and overall survival is poor in patients who have tumors which highly express the *LOX*. The *LOX* inhibition eliminates metastasis in breast cancer patients. *LOX* is required in metastatic growth to form a niche. *LOX* is required for hypoxia-associated metastasis. Although LOX inhibition has no significant effect on primary tumor growth, *LOX* was associated to significantly decrease the lung metastases and inhibited the liver metastasis [25]. *LOX* molecule is suggested to be a good therapeutic target in prevention and elimination of metastasis [25].

3.4 Brain metastasis

Brain/CNS (central nervous system) metastasis develops in 10–30% of metastatic breast cancer patients. Brain metastasis (BM) is detected as a complication that generally develops in the late stages of disease. Brain metastases develop after systemic emergence of metastases in the lungs, liver, and bone [26]. Two main primary tumors that do metastasis to the brain are lung and breast adenocarcinomas [18]. Brain metastases are associated with neurological disorders by affecting both the cognitive and sensory functions in addition to their association with highly poor prognosis.

Breast cancer is the most common cancer type where brain metastasis develops after lung metastasis. Lung and breast cancer-associated brain metastasis is more frequently detected than the primary brain tumors. Brain metastasis incidence has gradually been increasing in breast cancer patients. Due to the development of systemic therapies, many breast cancer patients live longer, but still in a way brain metastases may develop. Various factors were described for increased brain

metastasis risk in breast cancer patients. These factors may be reported as early age, poorly differentiated tumor histology (high grade), hormone receptor negativity, and metastasis in more than four lymph nodes. These factors were associated with the brain metastasis risk [26]. HER2-positive and TNBC patients have a higher risk of brain metastasis than the luminal-type breast cancer patients. Brain metastasis is detected in 30–40% of HER2-positive and triple negative breast cancer patients [26]. Brain metastasis in lung cancer generally develops within 2 years after the diagnosis of primary lung cancer, and brain metastasis in breast cancer is generally associated with the metastatic stage of the disease and develops 10 years after the primary diagnosis and after a successful treatment. However, brain metastasis in triple negative breast cancer patients develops in earlier periods. The development of brain metastasis in breast cancer was detected to be associated with Wnt, Notch, and EGFR pathways [27]. *CXCL12* that is expressed in the brain and *CXCR4* receptor located in the surface of the breast tumor cells block the cell signaling pathway together with *CXCR4* in brain metastasis. Breast cancer-associated brain metastasis generally develops in ~20–30% of breast cancer patients. Breast cancer-associated metastasis shows poor prognosis due to the lack of molecular therapeutic targets. The rate of detection of brain metastasis in HER2+ and triple negative breast cancer subtypes is 20–50%.

HER2 amplifications and mutations were frequently demonstrated in breast cancer and in breast cancers with brain metastasis [27]. There are no target-specific treatment options in the clinical practice generally in breast cancers that carry *BRCA1* and *BRCA2* gene mutation and triple negative brain metastasis. New molecular targets HER2, *EGFR*, *VEGFR*, *PARP*, *mTOR*, and *CDK-4/6* were discovered in the treatment of breast cancer with metastasis to the brain.

Brain metastasis is a multistep procedure with migration, intravasation, circulation, adhesion, extravasation, and brain microenvironment. Particularly the blood-brain barrier (BBB) is highly selective in the entrance of tumor cells and therapeutics to the brain microenvironment. In compliance with that, the cells to make a metastatic lesion in the brain have a specific clonal origin. This shows that a brain metastasis shared the common abnormalities with a metastasis ancestor cell, and the further abnormalities could only be present in only brain metastatic subclones. More frequent detection of *TP53* mutations in breast cancer with brain metastasis compared with the other breast cancers is an example. *COX2*, *EGFR*, and *HBEGF* were described as the extravasation stimulating factors through colonization in breast cancers with metastases to the brain and lung. The higher expression of the genes *CXCR4*, *PLLP*, *TNFSF4*, *VCAM1*, *SLC8A2*, and *SLC7A11* facilitates the development of brain metastases. In addition, the majority of snoRNAs and snRNAs have higher expression in breast cancer metastasizing to the brain [28].

3.5 Lung metastasis

Luminal breast tumors have the tendency to do metastasis to the bone; however, basal-like breast tumors mainly do metastasis to the lungs. The genes that are effective in the emergence of lung metastasis are generally associated with poor prognosis [29]. An epidermal growth factor receptor-ligand epiregulin (*EPR*) and the genes such as *COX2*, *MMP-1*, and *MMP-2* affect the tumor angiogenesis and facilitate the lung metastasis by reaching to the lung capillary vessels. The inhibition of *EGFR* and *COX2* minimizes the lung metastasis [30]. Protein deacetylase *SIRT7* was demonstrated to inhibit the development of lung metastasis of breast cancer cells by antagonizing the *TGF-β* signal [31]. An increased expression was reported in the genes *DSC2*, *TFCP2L1*, *UGT8*, *ITGB8*, *ANP32E*, and *FERMT1* that are associated with cell involvement and signal transduction in patients with lung metastasis of breast cancer [31].

Other genes except *PTEN* were detected to be overexpressed in the studies investigating the mechanism of lung metastasis. Although none of the described genes were found to be associated with previous metastasis, some of the encoded molecules were detected to have significant roles in the acquisition of proliferative and invasive characteristics to epithelial cells. The regulation of the epithelial-mesenchymal transition (EMT) is highly important in metastatic process. Integrins regulate the EMT by mediating the *TGF-β* signal activation [32]. *FERMT1* gene is known to be an effective gene in *TGF*-mediated epithelial-mesenchymal transition. Therefore, *FERMT1* gene is suggested to be associated with lung metastasis.

The decrease of the expression of a tumor suppressor gene *PTEN* was found to be associated with lung metastasis in a study [33]. *PTEN* is one of the main molecules which regulates the signaling pathways associated with reproduction, growth, cell viability, and cell migration and was detected to mutate in various different tumors. In addition, *PTEN* regulates the *EMT* in lung metastasis by affecting the cell viability and *CXCR4* chemotaxis. The biological indicators *EGFR* and *FOXC1* were demonstrated to be associated with each other and controlled the lung metastasis in breast cancer [33]. The survival rate of breast cancer patients with lung metastasis is very low despite the treatment options as chemotherapy, radiotherapy, and target-specific treatment against lung metastasis. Therefore, the development of new therapeutic strategies is significantly important for understanding the underlying mechanisms in lung metastasis.

A Notch signaling pathway receptor *Notch-1* was demonstrated to have a critical role in cell renewal, reproduction, and apoptosis of BCSC by regulating the epithelial-mesenchymal transition in breast cancer [34]. The abnormal activation of notch signaling pathway contributes to the breast cancer metastasis by primarily regulating the EMT and angiogenesis.

Wnt/β-catenin signaling has a significant role in the embryonic induction and tumorigenesis of the breast gland [35]. The nuclear localization and overexpression of β-catenin are an indicator of Wnt/β-catenin signal activation. Various clinical and laboratory studies showed that the abnormal activation of Wnt/β-catenin signaling was associated with poor prognosis in breast cancer patients and mainly increased in triple negative cancer subtype [36]. In addition, the Wnt-helper receptor *LRP6* was commonly overexpressed in highly aggressive triple negative breast cancer. Wnt/β-catenin signaling pathway contributes to the EMT and breast cancer metastases in addition to controlling the cell proliferation in breast cancer (**Table 1**).

Hedgehog (Hg) signaling pathway has a significant role in the development of ducts of the breast. In addition, Hg regulates the breast cancer stem cells and has a significant role in cancerogenesis [37]. Hg proteins regulate the breast cancer cell migration. Hg, Notch, and Wnt signaling pathways demonstrate joint behavior in tumor development and metastasis in cancer. These signaling pathways have significant roles in the development of breast cancer and lung metastasis.

Notch pathway	**Wnt pathway**	**Hedgehog pathway**
Uncontrolled growth	The self-renewal of breast cancer stem cells	*TGF-β*
The self-renewal of breast cancer stem cells	EMT	*CXCL12-CXC4*
Angiogenesis, EMT		
Formation of lung niches		
Development of lung metastasis		

Table 1.
The functioning of signaling pathways in breast cancer-associated lung metastasis.

Breast cancer is characterized with a separate metastatic pattern including the regional lymph nodes, bone marrow, lung, and liver. Chemokines are a group of small-molecular-weight protein which bind to chemokine receptors attached to G protein. Chemokines have a significant role in various pathological conditions such as cell migration, development, and inflammation. Binding of chemokines to receptors causes a structural change which activates the signaling pathways and promotes the migration. Chemokine and chemokine receptors have a critical role in identification of metastatic targets of tumor cells. Chemokines are divided into two groups in accordance with their functions as inflammatory chemokines and homeostatic chemokines. Inflammatory chemokines are induced by inflammation, and homeostatic chemokines are structurally expressed and have a role in homeostatic immune regulation [38].

Chemokines have a significant role in the progression of cancers [38] and have functions in tumoral growth, aging, angiogenesis epithelial-mesenchymal transition, and metastasis. The expression of chemokines and their receptors changes in malignity and then causes abnormal chemokine receptor signaling. This change stems from the inactivation of the tumor-suppressive genes or from the structural activation of oncogenes that have a role in the regulation of chemokines [38].

Chemokine receptors *CXCR4* and *CCR7* are highly expressed in human breast cancer cells, malignant breast tumors, and metastases [38]. In breast cancer cells, *CXCR4* or *CCR7* signaling mediates the actin polymerization and pseudopodia and then induces the chemotaxis and invasion.

The in vivo inactivation of *CXCL12*/*CXCR4* interactions significantly inhibits the metastasis of breast cancer cells to the regional lymph nodes and lungs [38]. *CXCL12*/*CXCR4* interactions also cause bone marrow metastasis of breast cancer cells.

Tumor cell migration and metastasis have various similarities with the leukocyte trafficking that are regulated by chemokines and their receptors. Cell trafficking-associated ligands *CXCL12/SDF-1α* and *CCL21/6Ckine* are highly expressed in the organs representing the first targets of metastatic breast cancer [38]. Malignant melanoma which has high skin metastasis and has a similar metastatic characteristic with breast cancer has high *CCR10* expression in addition to *CXCR4* and *CCR7* [38]. Therefore, both *CXCR4* and *CCR7* are highly critical molecules for cell trafficking and tissue homeostasis.

CXCL12 is the only ligand known for *CXCR4*. Metastatic breast cancers were demonstrated to selectively express CXCR4 and migrated to organs which highly express the ligand *CXCL12* that is also known as *SDF-1* [38]. *CXCR4* expression is known to be higher in malignant breast tumors than the levels in healthy breast tissues. *CXCL12* was highly expressed in organs such as the lung, bone, liver, and lymph nodes where the breast cancer cells preferred to do metastasis [38]. This showed that metastatic breast tumor cells selectively expressed *CXCR4*, and thus breast cancer cells which reached to organs have high *CXCL12* expression levels. In addition, the in vivo inhibition of *CXCR4-CXCL12* interactions was demonstrated to significantly decrease the metastasis of breast tumor cells to the lymph node and lungs [38]. Therefore, *CXCL12-CXCR4* signaling is suggested to be an important therapeutic target for metastatic breast cancer treatment.

CXCR4-CXCL12 receptor-ligand interactions in breast cancer allow the invading of tumor cells of neighboring tissues and for successful metastasis. The receptor-ligand interaction triggers the actin polymerization and facilitates the formation of pseudopodia. Thus, the invading of breast tumor cells of the neighboring tissues or distant tissue is induced or facilitated [39]. Chemokine *CXCL12* activates the chemokine receptor *CXCR4* in endothelial cell which supports the endothelial cell migration and growth [39]. The high expression of *CXCL12* in the lung, liver, and lymph nodes showed that these chemokines have a role in the metastasis of breast cancer cells for these anatomic regions.

CCL21 and its receptor *CCR7* have critical importance in the settlement of lymphocytes to secondary lymphoid organs. The primary breast cancer cells in lymph nodes and most metastatic cancer cells express *CCR7*, and there is an association between *CCR7* expression and lymph node metastasis. In addition, higher *CCR7* expression was demonstrated to be associated with poor prognosis and shorter survival [38].

Extracellular matrix (ECM) proteins tenascin-C (TNC), periostin (POSTN), and versican (VCAN) are highly important molecules in the formation of metastasis and have a critical role in the formation of breast cancer colonization in the lung tissue that has a tendency for metastasis. Tenascin-C, which is normally produced by fibroblasts, is also secreted by breast cancer stem cells. This abnormal expression of tenascin-C by breast cancer stem cells forms a niche in lung colonization and creates a metastasis-initiating effect. Periostin is a stromal factor that may bind to Wnt ligands and is effective in breast cancer metastasis [40].

Cancer-associated fibroblasts (*CAFs*) have a significant role in breast cancer metastasis by expressing the *Tiam1* and osteopontin in breast cancer tissue [40]. In addition, the expression of a *CAF*-associated protein thrombocyte-associated growth factor receptor (*PDGFRβ*) is highly associated with lung metastasis in breast cancer. In addition, CAFs increase the primary tumor growth through *TGF-β* and contribute to the development of lung metastasis-associated fibrous tissue in breast cancer [41]. Therefore, *CAF* is suggested to be a potential anticancer therapeutic target. The development of strategies targeting the microenvironment may be effective in the treatment or inhibition of breast cancer metastasis.

Because the lungs have a unique histological feature, cancer cell meets with high interstitial fluid pressure and thus supports the *PDGFRβ* expression when a cancer cell does metastasis to a small interstitial tissue between the alveoles. Lung metastasis is known to be associated with triple negative breast cancer.

As conclusion, the expression changes in these genes in breast cancer cells may be detected in bone, lung, brain, liver, and lymph node metastases. The studies revealed that there were important differences in metastatic behavior between breast cancer subtypes (**Table 2**). Therefore, the treatment of metastatic breast cancer must be performed by targeting the organ with metastasis, and the development of target molecules will form the future treatment protocols.

Luminal B, HER2+/ER/PR+ and HER2+/ER/PR, tumors do more metastasis to the brain, liver, lung, and bone than the luminal A tumors. Basal-like tumors do higher rates of brain and lung metastases. As demonstrated in **Table 2**, breast cancer cells do metastasis to the lung through triple negative breast cancer, basal, luminal B, HER2 molecular subtypes, the genes activated by growth factor receptors, matrix metalloproteinases, and the pathways of *COX2* and *LOX2* genes. Breast cancer cells with HER2+, luminal-HER2, triple negative breast cancer, and basal histologies primarily have a tendency to do metastasis to the brain. These molecular subtypes do metastasis to the brain with the effect of genes activated by growth factor receptors, matrix metalloproteinases, COX2, and chemokinesis. Clarifying the association of these signalings and genes with molecular subtypes suggests the significant new therapeutic targets for metastatic breast cancer treatment. The bone metastasis of luminal and HER2 breast cancer molecular subtypes is caused by growth factor genes and interleukins. Chemokine and integrin molecules that cause liver metastasis are more frequently detected in HER2+, ER+, luminal B, and luminal-HER2 molecular subtypes. BCR pathway proteins and CCN proteins, the genes responsible in Hg signaling pathway, cause lymph node metastasis in luminal type and HER2+ molecular subtypes [1].

Tissue	Lung	Brain	Bone	Liver	Lymph node
Molecular subtypes of breast cancer	TNBC Basal Luminal B HER2+	HER2+ Luminal-HER2 TNBC Basal	Luminal HER2	HER2+ ER+ Luminal B Luminal-HER2	Luminal HER2+
Molecular pathways and genes	**Growth factors** TGF-β EGFR VEGF **Matrix Metalloproteinases** MMP-1 MMP-2 **Chemokines** CXCL12 CXCR4 **BMP inhibitors** **Other factors** COX-2 LOX	**Growth factors** VEGF HBEGF **Matrix Metalloproteinases** MMP-9 MMP-1 **Chemokines** CXCR4 CXCL12 CCR7 CCL21 **Cytokines** CK5 **Notch pathways** **Wnt pathways** **Hg pathways** **Other factors** COX-2 LOX IL-8 COX-2 ICAM1 PTEN CAF	**Growth factors** IGF1 PGE2 TGF-β **Other factors** PDGF FGF2 IL11 IL-6 IL-1 OPN	**Chemokines** CXCR4 CXCL12 CCR7 CCL21 **Other factors** IL-6 N-cadherin E-cadherin LOX OPN VEGF TWIST WNT pathway ECM	**CCN proteins** **BCR pathways** **Hedgehog (Hg) pathway**

Table 2.
The organ-specific genes and signaling pathways effective in metastatic breast cancer.

Individualized target-specific appropriate treatment methods will be developed for metastatic breast cancer owing to the knowledge of the association of genes with each other that cause metastasis and the follow-up of the pathways where these genes gained function. There is an association between genomic differences and various gene expressions that cause poor prognosis in breast cancer. The gene expression profiles of primary tumors must be compared and associated with metastasis for describing and clarifying the tumor factors of metastatic breast cancer. The better understanding of the functioning of these genes will help to develop specific therapeutic approaches for metastatic breast cancer.

The molecules and genes on the pathways will be used in the diagnosis, prognosis and treatment response of metastatic breast cancer in the future. These effective molecules will be used as a tumor-specific indicator, and also detected in different biological materials like tissue, saliva, blood, serum, and urine in metastatic breast cancer. In addition, these genes may be used as therapeutic targets. The inactivation of these genes by inhibition or with biological antibodies through apoptosis is significantly important to resolve the tumor and metastasis. Different therapeutic strategies will be developed, and these molecules will be used in individualized treatment for inhibiting the tumor metastasis considering the associations between these genes, and chemokines, and integrins. The breast cancer molecular subtypes will be treated, and a progress will be enabled in the treatment of metastatic breast cancer with the development of molecular drugs which inhibit the active pathways or eliminate the pathway transition of the genes effective in metastatic breast cancer.

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Conflict of interest

The authors declare that they have no conflict of interests.

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