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*Review*

# Molecular Mechanisms of Breast Cancer Metastasis and Potential Anti-metastatic Compounds

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**Abstract.** *Throughout the world, breast cancer is among the major causes of cancer-related death and is the most common cancer found in women. The development of cancer molecular knowledge has surpassed the novel concept of cancer biology and unraveled principle targets for anticancer drug developments and treatment strategies. Metastatic breast cancer cells acquire their aggressive features through several mechanisms, including augmentation of survival, proliferation, tumorigenicity, and motility-related cellular pathways. Clearly, natural product-derived compounds have since long been recognized as an important source for anticancer drugs, several of which have been shown to have promising anti-metastasis activities by suppressing key molecular features supporting such cell aggressiveness. This review provides the essential details of breast cancer, the molecular-based insights into metastasis, as well as the effects and mechanisms of potential compounds for breast cancer therapeutic approaches. As the abilities of cancer cells to invade and metastasize are addressed as the hallmarks of cancer, compounds possessing anti-metastatic effects, together with their defined molecular drug action could benefit the development of new drugs as well as treatment strategies.*

Evidence shows that the dominant cause of death from the most common forms of cancer, including breast cancer, involves metastasis (1). Metastasis is listed as a hallmark of cancer (2) and is an important obstacle to the success of cancer management (3). Although the defined process and associated cellular mechanisms underlying metastasis have not been fully clarified, new technologies and molecular studies have much improved the knowledge of cancer biology and revealed novel anticancer drug targets. Metastatic breast cancer originates within the breast and disseminates from its primary site to nearby lymph nodes as well as other more distant sites throughout the body (4). After metastasis, breast cancer cells are found to be less responsive to chemotherapy. and patients with metastatic (stage IV) breast cancer can have a 5-year survival rate of as low as 22% (5).

In women, breast cancer is the most common cause of cancer death (6), and approximately 90% of breast cancer deaths are associated with metastasis of cancer cells. (7) Breast cancer can be categorized according to histological type, tumor grade, predictive markers [estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2)] and lymph node status. Breast cancer can be classified into five subtypes, which are shown in Table I (8).

The development of breast cancer is associated with several risk factors such as age, hormone status, family history and genetic predisposition. In terms of family history, patients who have first-degree female relatives who have been diagnosed with breast cancer will be at increased risk. The incidence of breast cancer also increases quickly with age, particularly in those aged over 50 years (9). The hormonal factor shows that women who experience menarche at an early age have a higher risk of developing hormone receptor-positive tumors (10). The risk is increased depending on the age at first birth; a higher age at first birth carries greater risks than being nulliparous (11). Moreover, evidence suggests that hormonal therapy also increases breast cancer risk (12). Interestingly, breast feeding protects against the

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Table I. *Classification of breast cancer.*

| Classification | Immunoprofile |
|---|---|
| Luminal-type A | $ER^+$, $PR^+$, $HER2^-$ |
| Luminal-type B | $ER^+$, $PR^+$, $HER2^+$ |
| HER 2 | $ER^-$, $PR^-$, $HER2^+$ |
| Basal | $ER^-$, $PR^-$, $HER2^-$ |
| Claudin-low | $ER^-$, $PR^-$, $HER2^-$ (Claudin-low) |

ER: Estrogen receptor; HER2: human epidermal growth factor receptor 2; PR: progesterone receptor.

development of some invasive breast cancer types (13). In recent years, knowledge of genetic predisposition to breast cancer has become clinically important (14). The mutation of genes such as breast cancer 1 (*BRCA1*), *BRCA2*, checkpoint kinase 2 (*CHEK2*), *ATM* serine/threonine kinase (*ATM*), and partner and localizer of *BRCA2* (*PALB2*) confer a higher risk of breast cancer (15). In addition, previous studies show that women who have a mutation in *BRCA1* and *BRCA2* have a severely increased risk of ovarian cancer (16).

## Stages of Breast Cancer

The stages of breast cancer, according to the American Joint Committee on Cancer (AJCC), can be divided into the TNM system: T: size of the breast tumor, N: extent of tumor spread to nearby lymph nodes and M: extent of tumor metastasis to other organs of the body. The earliest stage of breast cancer is called stage 0 or carcinoma *in situ*. In stage I, the tumor is small and has not spread outside the patient's breast. Stage II cancer is less than 2 cm in diameter and may also be found in some axillary lymph nodes. In stage III, the tumor found in the breast may be of any size, but the axillary cancer will not be equivalent to stage II. Moreover, the cancer has also spread to the chest wall and/or to the skin of the breast, causing dimpling, inflammation or change of breast skin color (17). Finally, in breast cancer stage IV, the cancer has disseminated to distant parts, such as the brain, lungs, plural or bone, as shown in Figure 1 (18).

## Metastasis in Breast Cancer

One of the hallmarks of cancer that is responsible for about 90% of cancer deaths is metastasis (19). Metastasis describes the spread of cancer cells from their original tumor to nearby tissues or other organs. Therefore, the importance of understanding the mechanism of the metastatic process and the factors that enhance metastasis, along with pathways that are involved in the process, has been broadly recognized.

Breast cancer is the second most malignant cancer in the U.S. (20); most patients do not die because of the primary tumor, but from the metastasis of the tumor to distant sites. Of all patients with breast cancer, 10-15% have aggressive disease leading to tumor spread to other organs within 3 years of developing the primary tumor (1). Breast cancer is classified as a heterogeneous disease (21), so it has a different nature of metastasis which makes it difficult to cure. Normally, primary breast cancer cells metastasize through blood vessels or lymph nodes into various distant organs such as the lungs, the liver and bones.

Generally, breast cancer cell dissemination comprises the common metastasis process found in many solid tumors as follows;

• Breast cancer cell detachment from extracellular matrix (ECM) and initiation of local invasion and migration: Metastasis starts with the disruption of the connection of the cell to ECM *via* cellular adhesion proteins such as integrins, leading to cancer cell dissociation from adjacent cells and the basement membrane. Cells with this enhanced invasive ability start to invade surrounding tissue with the help of proteolytic enzymes secreted to degrade the ECM and provide a route of invasion (22).
• Intravasation into the circulation: Cancer cells attach to the endothelial wall, then invade and move through the walls of lymph or blood vessels (23).
• Circulation: The tumor cells are spread *via* the blood or the lymphatic circulation to other organs. The cells must acquire anoikis resistance in order to maintain survival in an anchorage-independent manner (24).
• Arrest, adhesion, and extravasation at sites of metastasis: Tumor cells undergo cell-cycle arrest and adhere to capillary walls within target organs, before extravasating into the site of metastasis (25).
• Formation of metastatic tumor: Cancer cells possessing tumorigenic potential will proliferate to form small tumors (26).

As metastasis is a complex, multi-step process, metastatic cells require different properties to overcome hindrances, and most importantly, the ability to survive in detached conditions, to invade, and to generate new tumors. The disruption of any of these steps will stop the process of cancer metastasis (27). Moreover, cancer cells must resist the immune reaction which eliminates cancer cells and evade apoptotic signals in order to survive (28). If the tumor cells can complete these steps, they will produce secondary metastases (29).

## Mechanisms in the Metastatic Potential of Breast Cancer Cells

According to the hallmarks of cancer presented by Hanahan and Weinberg, 2000, the capability of a cancer cell to invade and metastasize is an important factor in determining the aggressive features of the disease and is a promising molecular target for drug discovery (2).

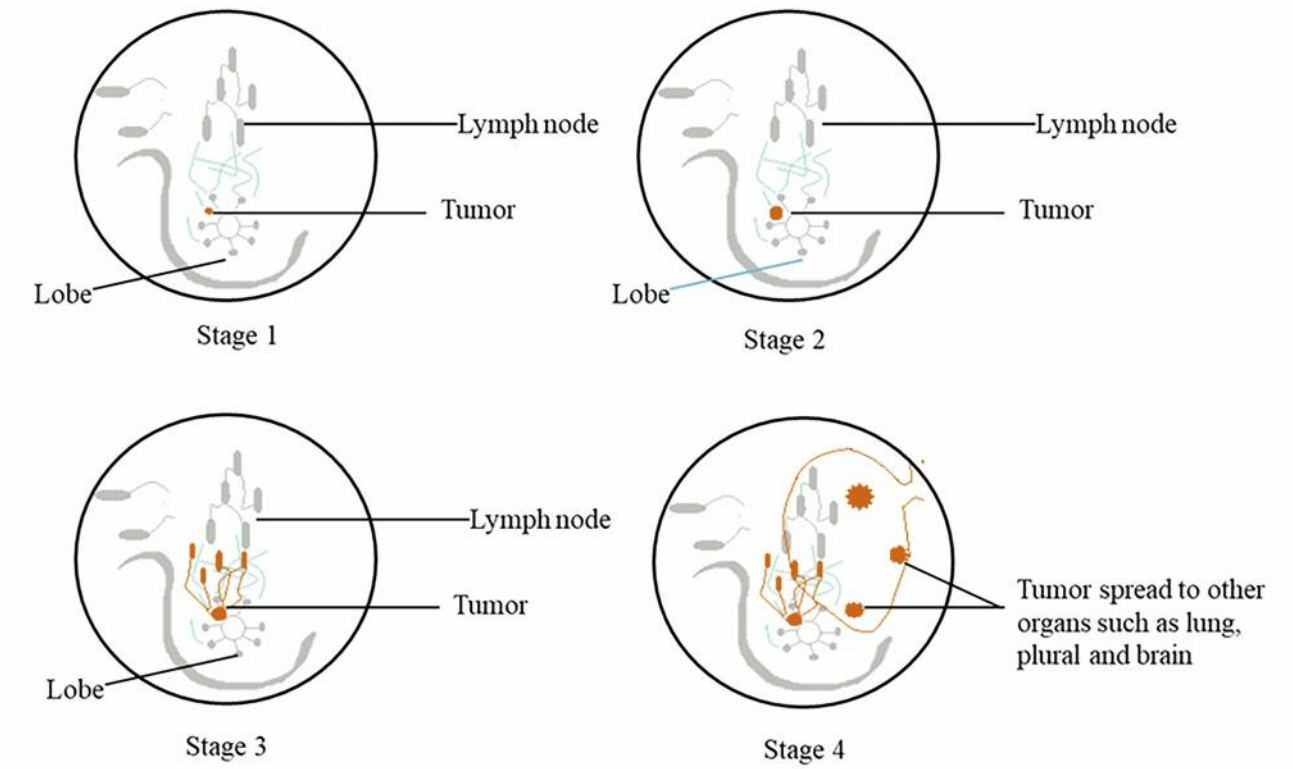



Figure 1. *Stages of breast cancer.*

Regarding cell to ECM interaction, the key proteins in cancer cell motility and survival are the integrins. Cancer cells attach to the ECM through the function of heterodimeric proteins known as the integrin family of extracellular matrix receptors. Integrins are composed of α and αβ subunits which transduce many signals from the ECM (30). Previous studies report that in poorly differentiated breast adenocarcinoma cells, expression of integrin α2β1 is reduced (31). Certain integrins such as integrin α3β1 have been associated with cancer cell invasion, metastasis, and activity of gelatinase β [metalloproteinase (MMP)-9] (32). The MMPs are a family of zinc-dependent endopeptidases which have the ability to degrade ECM component and also mediate proteolysis at the invadopodial front of invasive breast cancer cells (33, 34).

Likewise, E-cadherin was shown to play an important role in cell–cell adhesion and cancer metastasis (35). E-Cadherin is a cell–cell adhesion effector in normal epithelial tissues. Besides its role in normal epithelial cells, E-cadherin was shown to be involved in malignant transformation, tumor development, and tumor progression (36). A previous study showed that a decrease in the E-cadherin level in breast cancer is associated with increased metastatic potential (37). In addition, the down-regulation of such a protein is related to poor prognosis of patients with triple-negative breast cancer (38). Mutation in the E-cadherin gene (*CDH1*) leads to loss of invasion-suppressor function in lobular breast carcinoma (39).

Importantly, a decrease of E-cadherin expression is a key indicator of epithelial-to-mesenchymal transition (EMT), a cellular process that plays a critical role in cancer progression and metastasis (40). EMT of epithelial cancer cells can increase the ability of cancer cells to invade, metastasize and produce proteases involving the degradation of the ECM (41, 42). Furthermore, breast cancer cell lines exhibiting loss of E-cadherin and increased expression of N-cadherin expression were shown to have increased tumor cell invasion and metastasis activity (43, 44).

Regarding cell morphology, a cancer cell undergoing EMT exhibits the change from an epithelial adherent cell to a mesenchymal-like motile cell (45). The EMT process starts with losing expression of epithelial markers such as E-cadherin, occludin and cytokeratin. On the other hand, expression of mesenchymal markers such as vimentin and N-cadherin is found to increase. Specific transcription factors enhancing EMT, including twist-related protein (TWIST), SNAIL, SLUG, zinc finger E-box-binding homeobox 1 (ZEB1), and zinc finger E-box-binding homeobox 2 (ZEB2),

were shown to suppress E-cadherin expression in cancer cells (46). Furthermore, these EMT transcription factors control several signaling pathways such as transforming growth factor-β (TGFβ), Wingless/β-catenin, and the phosphatidylinositol 3' kinase serine/ threonine kinase (PI3K/AKT), and ample evidence has suggested that these pathways are associated with the poor prognosis of breast cancer (Figure 2).

TGFβ has been shown to function as an EMT inducer (47). TGFβ was shown to play a dual role in cancer regulation. During the early stages of tumor growth, TGFβ plays a tumor suppressor role by triggering growth arrest and eventually cell apoptosis (47). However, such tumor-inhibitory roles of TGFβ are suppressed during the process of tumor progression, and the exposure of the cancer cells to TGFβ at this stage enhances metastatic potential by converting epithelial phenotypes of the cells to mesenchymal-like phenotypes (48). For mechanistic approaches, TGFβ pathway has two types of signaling pathways: mothers against decapentaplegic homolog (SMAD) signaling pathway and SMAD-independent signaling pathway.

For SMAD-dependent signaling, when TGFβ ligand binds to type I and type II serine-threonine kinase receptors, TβRII phosphorylates TβRI to activate and regulate SMAD2 and SMAD3 by phosphorylation. Activated SMAD2 and SMAD3 form complexes with SMAD4 in the cytoplasm and is then translocated into the nucleus and interacts with transcription factors such as zinc finger protein (GLI1) to regulate gene transcription (49). Remarkably, SMAD2 and SMAD3 were found to be up-regulated in a mammary epithelial model, resulting in the induction of EMT (50). TGFβ signaling can be stimulated through non-SMAD signaling pathways such as the PI3K/AKT pathway. The PI3K/AKT/mammalian target of rapamycin (mTOR) signaling pathway regulates several cellular processes (51). Indeed, PI3K is a lipid kinase that triggers AKT activation. AKT, a serine/threonine kinase, functions to increase cell survival by activating several downstream effectors controlling cell proliferation and inhibiting apoptosis. Among such effectors, mTOR is a downstream target of the PI3K/AKT pathway that enables p70S6 kinase and 4E-binding protein-1 activation, resulting in cell proliferation (51). Activation of AKT/mTOR further controls glycogen synthase kinase-3β (GSK3β) and nuclear factor-κ B (NFκB). GSK3β has been shown to regulate several key cellular responses including apoptosis and cell cycle. An increase in NFκB can promote cell viability, proliferation, and malignant transformation (52). Accumulated evidence has suggested that the PI3K/AKT/mTOR pathway is often up-regulated in breast cancer (53). In addition, both SMAD-dependent and independent pathways were shown to control transcription factors mediating EMT, including TWIST, SNAIL, and SLUG (54). Taken together, above findings reveal attractive and promising targets for breast cancer therapy.

In relation to tumor growth, survival, invasion and metastasis, angiogenesis, the process of forming new blood vessels, has been intensively investigated (55-57), and the anti-angiogenesis therapeutic approach has gained much attention in the anti-cancer drug discovery area. In addition, data from clinical and experimental studies have indicated that breast cancer is a type of cancer which is angiogenesis-dependent (58). Several angiogenesis-potentiating factors have been identified and among them the expression of certain endothelial growth factors such as vascular endothelial growth factor (VEGF) shows the most potent activity, enhancing angiogenesis in various cancer types (59). VEGF directly induces endothelial cell proliferation and controls vascular permeability that aids the formation of new vessels.

From clinical studies, VEGF was linked with relapse-free survival, overall survival, or both (60). Patients with early-stage breast cancer with elevated expression of VEGF have a higher rate of recurrence or death in comparison to patients with low-angiogenic tumors (61), even if treated with conventional adjuvant therapy. In the process of new blood vessel formation, VEGF activates its receptor VEGFR1 and VEGFR2 in endothelial cells, resulting in the induction of endothelial cell motility, vascular permeability, cell survival, and proliferation (62, 63). Even though the definitive concept of VEGFR1 in cancer angiogenesis is still under-investigated, the impact of this receptor on cancer angiogenesis has been widely accepted (64-68). The increase in vascular permeability caused by VEGF signaling was shown to facilitate the spread of metastases in patients with cancer (24). Not only has the activity of VEGF in triggering angiogenesis been demonstrated and recognized as one factor potentiating breast cancer aggressiveness, but the VEGF signal was also shown to have several non-angiogenic functions (69). The VEGF pathway was shown in breast carcinoma cells to directly enhance cell survival through AKT and extracellular signal-regulated kinase (ERK) signals (70) (Figure 2). The activation of these keys in cellular survival pathway may help cancer cells to avoid apoptosis (71) and increase migration (61).

## Potential Anti-metastatic Compounds

Despite the high rate of cancer-related death in patients with metastatic breast cancer, therapeutic approaches in preventing metastasis are currently restricted. Taxol, isolated from the bark of the Pacific yew tree (*Taxus brevifolia*), was found to be very active in inducing cell-cycle arrest through blocking microtubule depolarization (72-74). Taxan™ has been used as a reference drug in comparing activity of potential new compounds for possible use against breast and ovarian cancer (72-74). Taxol was shown to be a potent agent in the treatment of metastatic breast cancer in phase II trials (75). Several plant-derived compounds have been

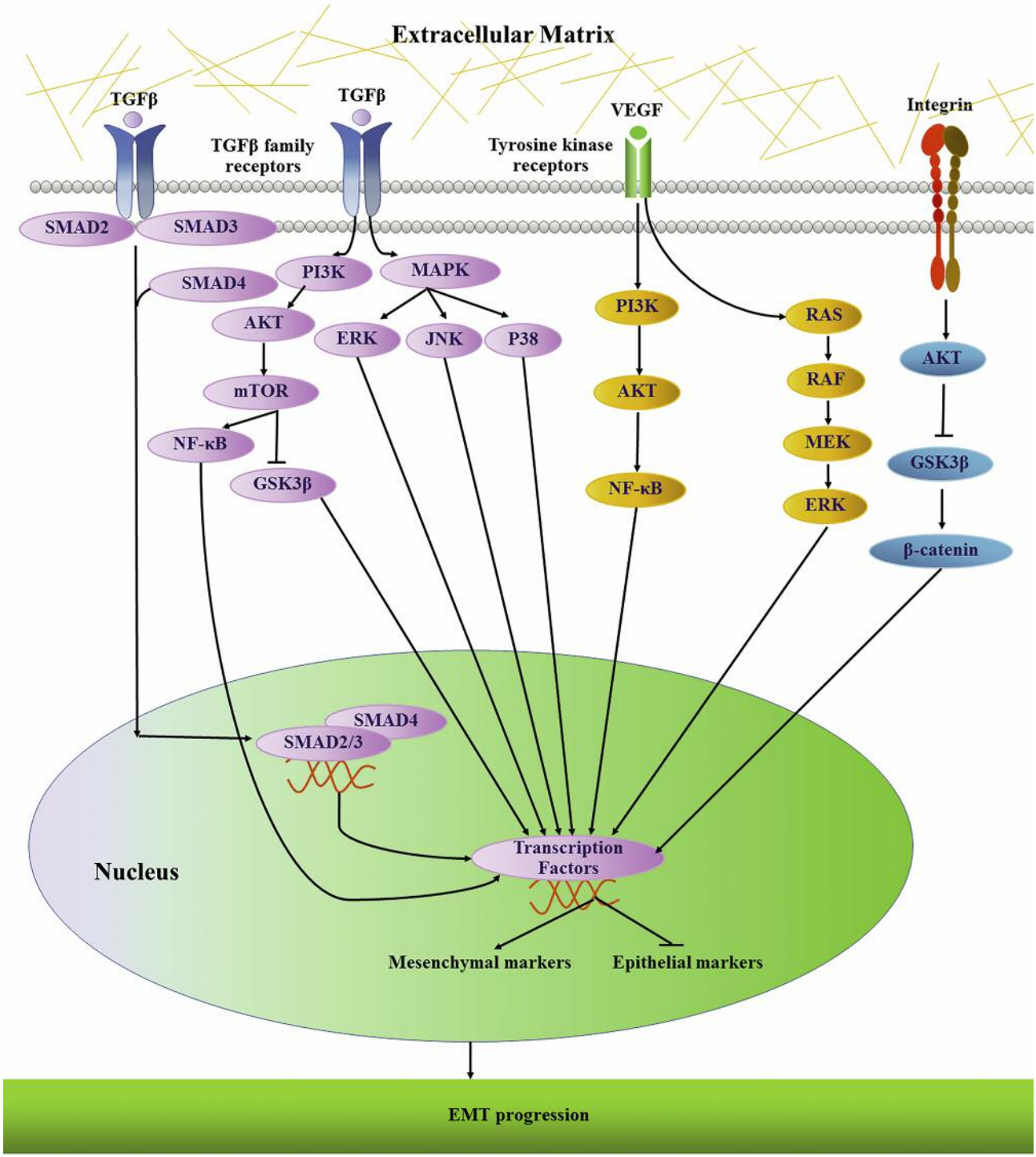



Figure 2. *Signaling in the progression of epithelial-mesenchymal transition (EMT). TGFβ: Transforming growth factorβ; VEGF: vascular endothelial growth Factor; SMAD: mothers against decapentaplegic homolog; PI3K: phosphatidylinositol 3-kinase; AKT: serine/threonine kinase; mTOR: mammalian target of rapamycin; NFκB: nuclear factor kappa B; GSK3β: glycogen synthase kinase 3β; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; MEK: mitogen-activated protein kinase kinase.*

Table II. *Natural product-derived compounds for anti-metastatic effects on breast cancer.*

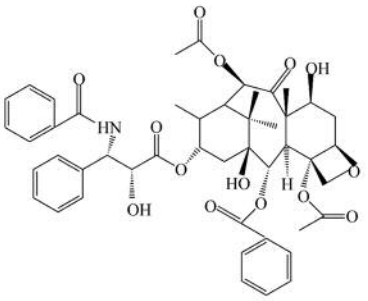
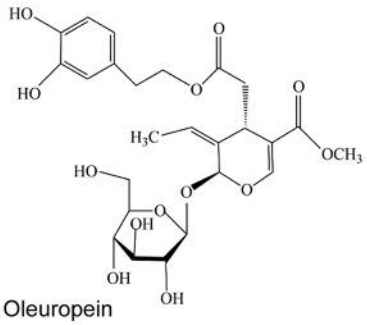
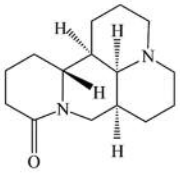
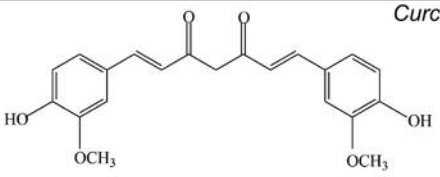
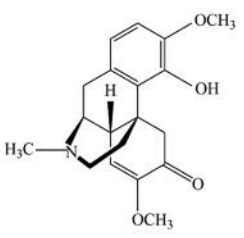
| Compound | Natural source | Origin, part used |
|---|---|---|
|  Taxol | *Taxus brevifolia* | Plant, bark |
|  Oleuropein | *Olea europaea* | Plant, whole body |
|  Matrine | *Sophora flavescens* Ait | Traditional Chinese herbal medicine, root |
|  Curcumin | *Curcuma longa* | Plant, root |
|  Sinomenine | *Sinomenium acutum* Rehd. *Et* Wils. | Traditional Chinese herbal medicine, stem |

Table II. *Continued*

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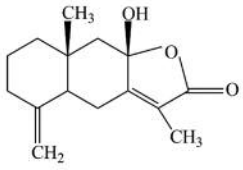
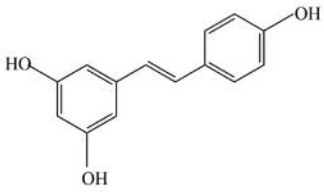
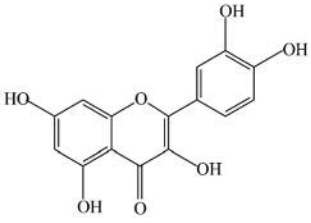
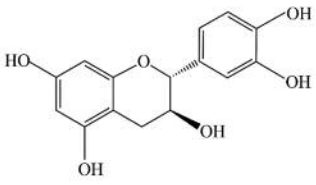
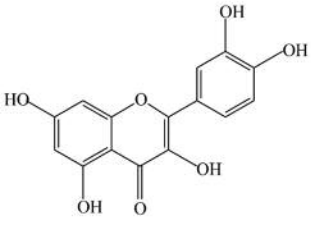
| Compound | Natural source | Origin, part used |
|---|---|---|
|  Codonolactone | *Atractylodes lancea* | Plant, rhizome |
|  Resveratrol | *Vitis vinifera* | Fruit, grape skin |
|  Quercetin | *Vitis vinifera* | Fruit, grape skin |
|  Catechin | *Vitis vinifera* | Fruit, grape skin |
|  Quercetin | *Vitis vinifera* | Fruit, grape skin |

tested and show potential for development for anticancer approaches in breast cancer (Table II).

Oleuropein, a major bioactive polyphenol of *Olea europaea*, the olive tree, exhibited strong anti-invasive effects when tested on breast cancer cells. Oleuropein has an anti-invasive effect by altering the expression pattern of invasion-related proteins. Breast cancer cells treated with oleuropein showed significantly higher levels of tissue inhibitor of metallopeptidase 1 (TIMP1) and TIMP3, while the treatment caused a dramatic decrease in the gene expression of *MMP2* and *MMP9* (76).

Matrine, a major bioactive compound isolated from *Sophora flavescens* Ait, a traditional Chinese tree, was investigated for potential pharmacological activities (77-82). The compound exhibited good anticancer profiles determined using several cancer cell lines (77). For breast cancer, matrine was tested on primary breast cancer-derived cells as well as metastatic cancer cells. Results showed that matrine inhibited the viability of cancer cells and induced apoptosis (81). Furthermore, *in vivo* models showed that administration of the compound significantly suppressed tumor growth of primary tumor formed in mice and inhibited their further metastasis to the lungs and livers (82). For the molecular mechanism, the cells isolated from the tumor of treated mice showed a significant decrease in the B-cell lymphoma 2 (BCL2)/ BCL2-associated X protein (BAX) ratio, and VEGF and VEGFR2 expression (82). The study suggests the potential of matrine to be further developed for breast cancer therapy.

Curcumin (diferuloylmethane), the main biologically active compound found in *Curcuma longa*, has long been known for anticancer potential. Curcumin has several anticancer properties such as anti-metastasis (83, 84), anoikis sensitization (85), chemotherapeutic drug sensitization (86), and direct apoptosis induction (87) in various types of cancer. It was also shown to prevent carcinogen-induced cancer *in vivo* (83). In MCF-7 breast cancer cells, curcumin was demonstrated to cause cell cycle arrest at $G_0/G_1$ and/or $G_2$/M phase and induce apoptosis (88). The cell cycle arrest cause by curcumin was found to be associated with the effect of this compound in up-regulating the cyclin-dependent kinases (CDK) inhibitor, $p21^{WAF/CIPI}$ and p53, and inhibition of transcriptional factor NFκB (89). Curcumin was shown to inhibit the assembly dynamics of microtubules and activate the mitotic checkpoint in a breast cancer cell line (90). Furthermore, treatment of cancer cells with curcumin resulted in the dramatic decrease of oncogenic and survival proteins including c-Jun, p38 kinase, $NH_2$-terminal kinase (JNK), ERK, and AKT (91). In relation to the signals that potentiate metastasis and angiogenesis, curcumin was shown to attenuate the induction of EGF in tumor cells (92, 93). Likewise, curcumin interrupted the signaling after activation of epidermal growth factor receptor (94). In addition, the combination of epigallocatechin gallate and curcumin showed a promising anticancer effect on ERα- breast cancer *in vitro* and *in vivo* models. In these processes, the regulation of VEGFR1 may play a key role in antitumor activities (95).

Sinomenine, is a compound isolated from *Sinomenium acutum* Rehd. et Wils. It was shown to have various pharmacological effects such as anti-inflammatory, anti-arthritis, and anticancer (96-98). Recent evidence has shown that sinomentine was able to inhibit cancer cell proliferation and mediated apoptosis by a mechanism involving the increase of cell-cycle arrest protein p21 and the decrease of the BCL2/BAX ratio, that in turn cause the release of cytochrome c from mitochondria, and activation of caspases (98). Interestingly, the chemically modified form of the compound, sinomentin hydrochloride can arrest the cell cycle of breast cancer cells at $G_1$ phase and induce ATM/ATR-CHK1/CHK2-mediated DNA damage (99). In terms of metastasis, sinomentin was shown to inhibit breast cancer cell invasion and metastasis by inhibiting EMT (100).

A natural compound from Atractylodes lancea named codonolactone was shown to attenuate the metastatic ability of breast cancer both *in vitro* and *in vivo* (101, 102). Besides various activities including anti-allergy, neuroprotective, and gastroprotective effects, codonolactone was shown to inhibit the metastatic potential of breast cancer cells by inhibiting TGFβ-mediated EMT. For the molecular mechanism, the treatment of the breast cancer cell with codonolactone inhibited the induction of runt-related transcription factor 2 (RUNX2) mediated by TGFβ. Treatment of the breast cancer cells with codonolactone significantly attenuate migratory and invasive activity of the cells (102).

Grape polyphenols, such as resveratrol, quercetin and catechin, were shown to inhibit growth of breast cancer and metastasis in nude mice with mechanisms involving suppression of AKT and mTOR activities, and activation of AKT/adenosine monophosphate protein kinase (AMPK), an inhibitor of mTOR (103).

As cancer-related mortality in breast cancer is tightly associated with metastasis, the mechanisms controlling such cancer cell dissemination have garnered increasing attention in both research and clinical fields. The cell adaptive response and molecular pathway that cancer cells use during metastasis are highly complex, however, several potential molecular targets to combat metastasis, including proteins enhancing survival, invasion, angiogenesis and EMT have been revealed. Understanding all of the key signals regulating breast cancer metastasis and the information of potential compounds affecting such pathways is beneficial for the development of drugs and strategies to inhibit cancer spread with greater efficiency, and improve the clinical outcome of patients with breast cancer.

## Conflicts of Interest

The Authors declare that there are no conflicts of interest regarding the publication of this article.

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